Potential for breeding sweet pepper adapted to cooler growing conditions

***A physiological and genetic analysis of growth traits in* Capsicum**

Promotor:	Prof. dr. ir. P. Stam Hoogleraar in de Plantenveredeling
Co-promotoren:	Dr. ir. R.E. Voorrips Onderzoeker Plant Research International, Wageningen
	Dr. ir. L.F.M. Marcelis Teamleider Gewasmanagement, Fysiologie en Modellering Wageningen UR Glastuinbouw
Promotiecommissie:	Prof. dr. R.F. Hoekstra, Wageningen Universiteit Prof. dr. ir. P.C. Struik, Wageningen Universiteit Dr. ir. E. Heuvelink, Wageningen Universiteit Dr. H. de Jong, Wageningen Universiteit Dr. ir. J.F.J.M. van den Heuvel, De Ruiter Seeds, Bergschenhoek

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

Potential for breeding sweet pepper adapted to cooler growing conditions

***A physiological and genetic analysis of growth traits in* Capsicum**

Erik A.M. de Swart

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 donderdag 7 juni 2007
des namiddags te 13:00 uur in de Aula

De Swart EAM (2007) Potential for breeding sweet pepper adapted to cooler growing conditions - A physiological and genetic analysis of growth traits in *Capsicum*

Ph.D. thesis, Wageningen University, Wageningen, the Netherlands
With references – with summaries in English and Dutch

ISBN: 978-90-8504-650-9

In the beginner's mind
there are many possibilities,
in the expert's mind
there are few.

Shunryu Suzuki

Ter nagedachtenis aan mijn moeder

Contents

Chapter 1

General introduction

GENERAL BACKGROUND

Sweet pepper (*Capsicum annuum* L.) is an important greenhouse crop in the Netherlands. In 2005, the total greenhouse production area in the Netherlands exceeded 1230 ha (CBS, 2006). In general, *Capsicum* requires relatively high temperatures to grow and produce fruit (Bakker and Van Uffelen, 1988; Poulos, 1993). Especially in the northern hemisphere, average yearly air temperatures are too low to grow sweet pepper in open fields or unheated greenhouses. Low temperatures are not only hampering plant growth (Ottosen *et al.*, 2001; Pet, 1983) and fruit quality (Gruda, 2005; Ottosen *et al.*, 2003), but also reduce the number of viable pollen grains per flower (Pressman *et al.*, 1998) and fruit set (Pet, 1983). High temperatures, on the other hand, also reduce fruit set and pepper productivity in sweet pepper (Erickson and Markhart, 2001).

To be able to produce sweet peppers during the whole year in the Netherlands, the plant is grown in greenhouses heated by natural gas. As a consequence, the energy input needed to grow sweet pepper is very high, especially during winter time: on average, a greenhouse sweet pepper crop requires approximately 42 m^3 natural gas per m^2 per year (Anonymous, 2004). The Dutch horticultural sector as a whole is responsible for about ten percent of the natural gas consumption in the Netherlands and therefore is a large producer of CO_2 (Anonymous, 2004).

In 1998, the Netherlands signed the Kyoto Protocol. This protocol requires the Netherlands to reduce its emissions of greenhouse gasses by about 6% - as compared to the 1990 levels - by the year 2008–2012 (Anonymous, 1998). Moreover, the greenhouse horticulture sector and the Dutch authorities specified a target of a 65% improvement in the energy efficiency index - the input of primary fuel per unit of product - by 2010 as compared to the reference year of 1980 in the Greenhouse Horticulture Covenant (Anonymous, 1997). Social and political demands concerning these issues cause the horticultural sector to search for ways to reduce the emission of greenhouse gasses and improve the energy efficiency in horticultural production.

Scientific research has contributed in several ways to the increase of energy efficiency in Dutch horticulture. Technical measures such as materials covering greenhouses (Swinkels *et al.*, 2001), the use of thermal screens (Bot, 2001; Lund *et al.*, 2006; Van der Knijff and Benninga, 2003), improvements in heating technology (De Zwart and Bot, 1997), low energy dehumidification systems (Campen and Bot, 2001; Van der Knijff and Benninga, 2003) and heat storage (Van der Knijff *et al.*, 2006) have been shown to reduce energy use. Furthermore, dynamic climate control - using the ability of a crop to compensate for temperature changes within a limited period – has been shown to lead to a significant energy reduction compared to standard climate conditions (Dieleman *et al.*, 2005; Körner *et al.*, 2004; Körner and Challa, 2003; Lund *et al.*, 2006; Ottosen *et al.*, 2001, 2003; Sigrimis *et al.*, 2000).

A simple approach resulting in energy use reduction is to lower the greenhouse temperature (Elings *et al.*, 2005). An obvious disadvantage of this approach is that it leads to a decrease in production when the plants used are not adapted to these lowered temperatures. One way to get around this is to breed cultivars that are tolerant to lowered temperatures without yield losses (Jauhar, 2006). In several crops the presence of genetic variation for tolerance to lowered temperatures has been demonstrated (Foolad and Lin, 2001; Hekneby *et al.*, 2006; Oleksyn *et al.*, 1998; Van der Ploeg and Heuvelink, 2005, 2006), indicating that there is a perspective for breeding for such plants. In *Capsicum* little information is available on low temperature tolerance. The prospects for improvement of cultivated species of *Capsicum* in this respect through breeding are good: the genetic diversity available within the various domesticated species has been little exploited and has certainly not yet been exhausted (Pickersgill, 1997).

In this thesis the possibility is investigated to breed for sweet pepper cultivars which are able to grow under lower temperatures than current standard cultivation temperatures, without a significant loss in production. Therefore, the physiology of growth and development and genetics of these traits in *Capsicum* are studied in the following chapters.

GENUS *CAPSICUM*

Capsicum belongs to the Solanaceae or nightshade family (Heywood, 1978). The genus consists of more than 20 wild species and five domesticated species (Bosland, 1994; Poulos, 1993). These domesticated *Capsicum* species are *C. annuum* L., *C. baccatum* L. , *C. chinense* Jacq, *C. frutescens* L. and *C. pubescens* R. & P. Different opinions exist on the question whether *C. annuum*, *C. chinense* and *C. frutescens*, all belonging to the *C. annuum* complex (*C. annuum*, *C. chinense*, *C. frutescens*, *C. chacoense* and *C. galapagoense*) are distinct species, based on their distinguishing characteristics (Pickersgill, 1971, 1988). *C. baccatum* and *C. pubescens* certainly are two distinct species (Baral and Bosland, 2004; Heiser and Smith, 1953; Onus and Pickersgill, 2004; Smith and Heiser, 1957).

The genus *Capsicum* originates from South and Central America (Bosland and Votava, 2000; Heiser and Smith, 1953) and *Capsicum* species occur in a wide range of different habitats. In general, they are considered to be warm season adapted, day neutral plants (Poulos, 1993) that are cold sensitive (Simon *et al*., 1984). Reported growth temperatures in the natural habitats of *Capsicum* vary between 7° and 29°C (Simon *et al*., 1984), although for most *Capsicum* species the optimal temperature for productivity is between 18° and 29°C (Poulos, 1993). *C. pubescens* is the only *Capsicum* species that is adapted to cooler growing conditions (4° to 21°C), but it does not tolerate frost (Bosland, 1996). Furthermore, some varieties of *C. baccatum* are able to germinate at temperatures between 10° and 13°C (Randle and Honma, 1980). *Capsicum* species naturally occur in regions with an annual precipitation between 0.3 and 4.6 m and grow best in well-drained, sandy or silt-loam soil with a pH between 4.3 and 8.7 (Simon *et al*., 1984). Some species, for example *C. chinense* and *C. fructescens* are confined to lowland areas, whereas *C. pubescens* is found only in highlands of South and Central America (Heiser, 1964; Heiser and Smith, 1953; Pickersgill, 1997). *C. annuum* is found in a wide range of altitudes (Heiser, 1964).

Over the years, man has tried to improve the production and quality of domesticated *Capsicum* varieties by crossing the different *Capsicum* species.

Crosses between *C. annuum*, *C. chinense*, *C. frutescens* and *C. baccatum* have a good chance of producing fertile F_1's (Pickersgill, 1971, 1988; Smith *et al.*, 1987) but *C. pubescens* will not hybridise with the other four species (Smith *et al.*, 1987). The latter is caused by unilateral incompatibility. Although direct hybridisation between *C. pubescens* and most members of the *C. annuum* complex is impossible, Zijlstra *et al.* (1991) suggested that *C. chacoense* may prove useful as a bridge species between *C. annuum* and *C. pubescens*. Furthermore Molchova and Michailova (1982) were able to cross plants of *C. annuum* and *C. pubescens* by using a tetraploid accession of *C. annuum*. Finally, Pickersgill (1988) speculated that the unilateral incongruity between *C. pubescens* and members of the *C. annuum* complex could possibly be overcome by using embryo rescue.

GROWTH AND DEVELOPMENT, PHYSIOLOGY AND MORPHOLOGY

Many plant species are able to grow in a large range of different habitats and to adapt to local environmental conditions. Plants that originate from unfavourable environments usually have a low inherent relative growth rate (RGR; increase in dry mass per unit dry mass per unit time) under non-limiting growth conditions, whereas plants that naturally occur in favourable habitats have an inherently high RGR (Grime and Hunt, 1975; Poorter and Remkes, 1990). Grime and Hunt (1975) suggested that RGR was the selection criterion for adaptation to stress conditions. Others suggested that the target for selection was not RGR itself, but one of the growth related traits linked with RGR (Lambers and Poorter, 1992).

RGR can be divided into two components or growth related traits. The first is a physiological trait called the net assimilation rate (NAR; increase in dry mass per unit leaf area per unit time; Evans, 1972). NAR is the net result of carbon gain due to photosynthesis and carbon losses, mainly due to respiration (Poorter, 1989a). The second characteristic, the leaf area ratio (LAR; the leaf area per unit of total plant dry mass) is a morphological component. LAR can further be divided into specific leaf area (SLA; leaf area

per unit leaf dry mass) and leaf mass fraction (LMF; the fraction of total plant dry mass allocated to the leaves; Evans, 1972). In short:

RGR = NAR • LAR = NAR • SLA • LMF

In general LAR and, more specifically, SLA, is the most important factor to explain the inherent variation in RGR between species; NAR seems to be of only secondary importance (reviewed by Poorter, 1989a; Poorter and Van Der Werf, 1998). Some studies, however, show that NAR is at least of equal importance as LAR in determining differences in RGR between or within species (Atkin *et al.*, 1996; Biere, 1996; Garnier, 1992; Reiser *et al.*, 2002; Shipley, 2006; Si and Thurling, 2001a; Van der Ploeg *et al.*, 2005; Verhoeven *et al.*, 2004; Villar *et al.*, 1998).

As stated, one of the environmental conditions to which plant species can adapt is temperature (Beadle *et al.*, 1985). Especially in the early stages of growth plants are reacting to changes in temperature (Thorvaldsson and Martin, 2003). Plants have an optimal temperature for growth (Friend and Helson, 1976; Hunt and Halligan, 1981; Mitchell, 1956) and exposing plants to lowered, suboptimal temperatures often reduces RGR (Atkin *et al.*, 2006; Cavaco *et al.*, 2003; Friend and Helson, 1976; Xiong *et al.*, 2000). The components of RGR (see above) also react to varying temperatures. The relation of NAR to temperature can be described as a curve with an optimum close to the optimal growth temperature (i.e., the temperature where the highest RGR is reached; (Hunt and Halligan, 1981; Tjoelker *et al.*, 1998), or a temperature close to the natural growth temperature (Bednarz and Van Iersel, 2001; Soldati *et al.*, 1999; Van Iersel and Lindstrom, 1999). Growing plants at higher or lower temperatures compared to the optimal growth temperature will influence NAR negatively. The influence of suboptimal temperature on SLA is not unambiguous: in some studies, SLA decreases at lowered temperatures (Hekneby *et al.*, 2006; Loveys *et al.*, 2002) while other studies show no effect of temperature on SLA (Hovenden, 2001). Similarly, Atkin *et al.* (2006) concluded that the influence of temperature on SLA depends on which species is being investigated and the temperature regimes at which the plants are grown. LMF seems to be insensitive to variation in temperature, at least in

cases were the above and below ground temperature are the same (Bruhn *et al.*, 2000; Loveys *et al.*, 2002; Tjoelker *et al.*, 1998).

In *Capsicum* little information is available on the influence of suboptimal temperature on growth and development. Information on the influence of both day and night temperature on the relationship between RGR and growth-related traits has been limited to studies using only one sweet pepper accession (Nilwik, 1980a, b, 1981a, b). These showed that RGR (Nilwik, 1981b), NAR (Nilwik, 1981a) and SLA (Nilwik, 1980a, b) were reduced under lowered temperatures, whereas LMF was unaltered (Nilwik, 1980a, b). The influence of suboptimal temperature on other morphological plant traits has been described more elaborately: values for dry mass, fresh mass, leaf area, plant height, number of leaves and internode length decrease when *Capsicum* plants are exposed to lowered temperatures (Bakker and Van Uffelen, 1988; Mercado *et al.*, 1997; Nilwik, 1980a; Pressman *et al.*, 2006; Si and Heins, 1996). Other studies only focus on the effects of lowered night temperature on *Capsicum* growth and development (Deli and Tiessen, 1969; Rylski, 1972), flowering (Bhatt and Srinivasa Roa, 1993), fruit setting (Bhatt and Srinivasa Roa, 1993) and number of fruits (Deli and Tiessen, 1969).

Most of the work described in this thesis is based on the ability of different *Capsicum* species to adapt to lowered temperatures. We have defined this adaptation as the relative difference in RGR between plants grown at different temperature conditions. Using growth analysis we will describe the variation in RGR within a wide range of wild and cultivated *Capsicum* accessions. The presence of and hence the possibility to breed for lowered temperature tolerance in this genus will be determined by comparing the variation in RGR as a result of decreasing growth temperatures. Physiological and morphological plant traits, which are related to RGR and relevant for practical breeders, will be identified in the next chapters and the inheritance of these traits will be analysed. Measurements are performed on plants in the vegetative growth phase since especially young plants are sensitive to changes in temperature (Thorvaldsson and Martin, 2003). Furthermore, I assume that RGR in *Capsicum*, as in *Brassica* (Si and Thurling, 2001a), gives a good prediction of later dry mass development and probably of generative growth and production.

GENETICS

A Quantitative Trait Locus (QTL) is a chromosomal region containing a locus (or loci) that affects a quantitative trait. QTLs are detected by means of associations between molecular markers, which have been located on a linkage map, and phenotypic traits (reviewed by e.g. Xu, 1997). QTLs give an approximate position of the genes underlying quantitative traits on the genome (Price, 2006; Salvi and Tuberosa, 2005). The opportunity to apply genetic markers to improve crops allows breeders to precisely transfer beneficial alleles into agricultural strains (Phillips, 2006). Furthermore, the fundamental process of selection has been improved by our better understanding of gene action and better methods to select plants (reviewed by Koornneef and Stam, 2001; Baenziger *et al.*, 2006). These genetic tools, however, do by themselves not lead to quantitative understanding of crop performance. Therefore, the need to connect genetics to whole plant function, development and yield and to evaluate germplasm under field conditions still remains (Boote and Sinclair, 2006).

QTLs for many important agronomic traits such as leaf area (Byrne *et al.*, 1997; Mansur *et al.*, 1996), plant height (Hittalmani *et al.*, 2003; Hittalmani *et al.*, 2002; Mansur *et al.*, 1996; Paran *et al.*, 1997), yield (Hittalmani *et al.*, 2003; Hittalmani *et al.*, 2002; Mansur *et al.*, 1996), cold tolerance (Andaya and Mackill, 2003), fruit quality and fruit shape (Serquen and Staub, 1997; Van der Knaap and Tanksley, 2001) have been identified in various species. The first publications on attempts to identify QTLs for RGR and growth related traits stem from the late nineties of the last century (Courtois *et al.*, 2000; Yin *et al.*, 1999b). Although Courtois *et al.* (2000) suggested that QTLs of RGR may be difficult to detect because that trait is controlled by several loci on the genome, QTLs for RGR were identified later on in a number of species, including *Hordeum spontaneum* (Elberse *et al.*, 2004; Poorter *et al.*, 2005; Van Rijn, 2001), *Aegilops* (Ter Steege *et al.*, 2005), *Salix* (Weih *et al.*, 2006) and *Arabidopsis* (El-Lithy *et al.*, 2004). In addition, QTLs were identified for traits underlying RGR: in *H. spontaneum* QTLs were found for NAR (Poorter *et al.*, 2005), LAR and LMF (Elberse *et al.*, 2004; Poorter *et al.*, 2005). Both in barley

(Yin *et al.*, 1999a) and in maize (Hund *et al.*, 2005) QTLs for SLA were recognized. In maize, these QTLs were temperature dependent, indicating that SLA was controlled by different genes at suboptimal and optimal temperatures (Hund *et al.*, 2005).

In *Capsicum*, identified QTLs for plant morphology are limited to those for plant height (Ben Chaim and Paran, 2000). For RGR and growth related traits no QTL studies have been published. Many genetic studies of fruit characteristics such as fruit weight (Rao *et al.*, 2003), fruit shape (Ben Chaim *et al.*, 2001a; 2003a; b) fruit pungency (Blum *et al.*, 2003) and fruit colour (Huh *et al.*, 2001; Lefebvre *et al.*, 1998; Popovsky and Paran, 2000) have been reported in *Capsicum*. Furthermore, many reports focus on disease resistance (Ben Chaim *et al.*, 2001a; Caranta *et al.*, 1997; 2002; Lefebvre *et al.*, 2003; Quirin *et al.*, 2005; Thabuis *et al.*, 2004; Voorrips *et al.*, 2004) and pest resistance (Djian-Caporalino *et al.*, 2001). In this thesis we will identify QTLs for plant morphology and growth related traits that can be used in breeding programmes.

AIMS OF THE THESIS

The work described in this thesis can be considered as a quest to determine the possibilities to breed for lowered temperature tolerant and thus energy efficient sweet pepper cultivars. To be able to do so, information is needed on the physiology of growth and development and genetics of *Capsicum*. Therefore the aims of this thesis are:

- To determine the variation in RGR and its underlying physiological and morphological traits in a group of ten wild and cultivated *Capsicum* accessions of four *Capsicum* species at two contrasting temperatures.
- To identify *Capsicum* accessions which show only a small decrease in RGR at lower temperatures and to identify the growth related traits that contribute to this adaptation.
- To simplify selection for RGR in breeding programmes by identifying physiological and morphological traits that are closely linked to RGR under various temperature regimes and which are indicative for RGR.

- To obtain information about the amount of low temperature tolerance present in the genus *Capsicum* in relation to this variation present in sweet pepper.
- To identify QTLs with an effect on plant morphology, growth and development that could be used in breeding programmes for better growth under lowered temperatures.

OUTLINE OF THE THESIS

Chapter 2 of this thesis describes a method for non-destructive estimation of leaf area in *Capsicum* which will be used to perform repeated non-destructive measurements of leaf area on genetically unique F_2 and F_3 plants (see Chapter 5). Chapter 3 describes the variation in adaptation to lowered temperatures in a group of ten wild and cultivated *Capsicum* accessions of four *Capsicum* species. This variation is determined performing a growth analysis at two contrasting temperature regimes; variation in RGR, growth related traits and their mutual relationships are analysed. In Chapter 4, the relationship between RGR, relative leaf growth rate (RLGR) and a number of morphological plant traits (leaf, stem and total fresh- and dry mass, plant height, leaf area, number of leaves) is studied to identify which trait can be used to simplify the selection for RGR in breeding programmes. Furthermore, a comparison is made between the variation in the ability to adapt to lowered temperatures in cultivated sweet pepper varieties on the one hand and the variation found in the *Capsicum* accessions studied in Chapter 3 on the other hand. The aim of Chapter 5 is to identify QTLs with an effect on plant morphology, growth and growth related traits in *C. annuum*. Therefore, an intraspecific F_2 population of the *Capsicum* accessions which exhibited large differences in phenotypic characteristics (unpublished pilot studies and Chapter 3) will be measured and analysed. A genetic map of this F_2 population is constructed and QTL analysis is performed. The effect of the QTLs identified is verified in a group of F_3 lines selected on the homozygous presence of allele of the QTL for RLGR of one of the parental plants. Finally, Chapter 6 integrates and discusses the main results of chapters two to five.

Chapter 2

Non-destructive estimation of leaf area for different plant ages and accessions of Capsicum annuum *L.*

Erik A.M. de Swart, Remmelt Groenwold, Jos Kanne, Piet Stam,
Leo F.M. Marcelis, Roeland E. Voorrips (2004)

Journal of Horticultural Science & Biotechnology 79: 764-770

ABSTRACT

Accurate measurements of leaf area are important for agronomic and physiological studies. To be able to perform repeated measurements of leaf area on single (genetically unique) plants, a method was developed to estimate leaf area from non-destructive measurements in *Capsicum annuum* L. independent of plant age and accession. Leaf length (L), width (W), position (leafno) and area of individual leaves were measured on 160 plants of four *Capsicum* accessions with different leaf shapes at different plant ages. Leaf area could be predicted from the product of length and width (α•L•W), but this model could not account for changes in leaf shape during development of individual leaves and was dependent on both plant age and accession. The model became independent of plant age and accession when leaf width terms (W^2, W) were added. Validation on leaves measured in a second experiment containing three accessions and 200 genetically unique F_3 plants showed that the relation between calculated and measured area was very high. The model could still be refined by addition of the leaf position terms (R^2 = 0.996). Even when the length and width of only 25% of the leaves were measured, total plant leaf area was predicted well using the model with both leaf width and leaf position terms. Therefore, the models including the leaf width terms (W^2, W) in addition to the L•W term are useful tools in physiological research and breeding in *Capsicum*.

INTRODUCTION

Plant leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis and plant productivity (Goudriaan and Van Laar, 1994). Leaf area can be measured either by destructive or non-destructive measurements. Accurate, non-destructive measurements permit repeated sampling of the same plants over time and have the advantage that biological variation can be avoided. Especially when unique plants, for example in genetically segregating populations are used, non-destructive measurements are of great value. A common approach for non-destructive leaf area estimation is to develop ratios and regression estimators by using easily measured leaf parameters such as length and width (Schwarz and Kläring, 2001). Various combinations of measurements and various models relating length and width to area have been utilised in for example maize (Stewart and Dwyer, 1999), squash (NeSmith, 1992), bitterleaf (Ajayi, 1990), grape (Montero *et al*., 2000), sunflower (Bange *et al*., 2000), muskmelon (Panta and NeSmith, 1995) and *Capsicum* (Bakker, 1989; Kläring *et al*., 1996; Ray and Singh, 1989; Shivashankar *et al*., 1986). The accuracy of the predictions however, is dependent on the variation in leaf shape within a single plant and between accessions.

Plants produce several different types of leaves during development. The first few true leaves produced are usually smaller, simpler, and anatomically different from leaves produced later in development (Poethig, 1997). Change in shape and size of successive leaves on a plant are related to physiological changes associated with increasing age of the plant (Esau, 1965), interaction between the shoot apical meristem and the developing leaf primordia (Byrne *et al*., 2001), genetically regulated programs of shoot maturation and a variety of environmental factors (Poethig, 1997). One of the leaf shape parameters is the length:width ratio. Verwijst and Wen (1996) found that in Salix the length:width ratio changed with leaf size and differed between different types of shoots. Sugiyama and Oozono (1999) showed that in lettuce this ratio of individual leaves decreased with time and eventually became constant. Comparable results were found in red spruce (Day *et al*., 2001).

Consequently the ratio between leaf area and the product of length and width changes with plant age (Marshall, 1968). Persaud *et al.* (1993) suggested that in pearl millet this ratio should be calculated for each leaf position and growth phase of the plant.

We needed a good model for non-destructive leaf area estimation for use in physiological and genetic studies on the vegetative growth phase of (genetically unique) *Capsicum* plants. Therefore, the aims of this study were 1) to develop a model for leaf area prediction from simple non-destructive measurements in *Capsicum* that was able to accommodate the effect of changes in leaf shape during development and differences in leaf shape between accessions and which could be used for *Capsicum* plants of all accessions and ages without recalibration, 2) to assess the robustness of the model on an independent set of data from (partially) other accessions grown under different environmental conditions, and 3) to examine the minimum number of leaves that has to be measured to accurately predict total plant leaf area.

MATERIALS AND METHODS

Plant cultivation

Capsicum annuum L. plants were grown in a glasshouse in Wageningen (The Netherlands, latitude 52 °N). Seeds were sown in rock wool plug trays (25 x 25 x 40 mm plug^{-1}), moistened with water, and covered with fine vermiculite. Seedlings were transferred to rock wool cubes 17 days after sowing (d.a.s.) and placed on an ebb-flow system. After transfer, plants were irrigated with nutrient solution (EC = 2.1 dS m^{-1}; pH = 5-6) containing (in mM): 0.5 NH_4, 6.75 K, 5.0 Ca, 1.5 Mg, 15.5 NO_3, 1.75 SO_4, 1.25 P, 0.015 Fe, 0.01 Mn, 0.005 Zn, 0.03 B, 0.00075 Cu and 0.0005 Mo. The actual average day temperature in the glasshouse was 22.5 °C (16 hours) and the night temperature was 18 °C (8 hours). Artificial HPI-T light (32.6 ± 2.2 µmol m^{-2} s^{-1} PAR) was added during 16 hours per day. Relative air humidity varied from 50 to 70%.

Capsicum plants used in the experiments develop a single stem with 9 to 12 leaves. The main stem ends with one or two flowers and branches into

two or three side branches. At each (first or higher order) branch one leaf develops and the branch terminates in a flower and divides into two or three higher order branches. In this experiment, two first order branches were retained. Subsequently, the largest of each higher order branch was retained, while the smallest one was removed above the first leaf. All other shoots were removed twice a week. This pruning strategy corresponds largely to common practice of commercial growers.

Calibration experiment

Plants from four *C. annuum* accessions were grown from November 2001 to January 2002. Accessions were selected based on their different leaf shape (Anonymous, 1995). 'Jatilaba' (RIV Lembang, Indonesia) is an Indonesian hot pepper with lanceolate shaped leaves. 'Bruinsma Wonder' (CGN19226) and 'Nassau F_1' (Rijk Zwaan) are both Dutch sweet peppers with deltoid leaves. F_1 '(Jatilaba x Bruinsma Wonder)' has an intermediate leaf shape.

During growth the average daily global radiation outside of the glasshouse was 2.5 MJ m^{-2} d^{-1}, ranging from 0.3 to 6.7 MJ m^{-2} d^{-1}. The light transmission of the glasshouse was about 50%. No CO_2 was supplied. As plants grew larger and needed more space, plant density was gradually decreased from 15 plants m^{-2} at 17 d.a.s. to 6 plants m^{-2} at 49 d.a.s.

Measurements started when all plants had at least two true leaves longer than 2.0 cm (31 d.a.s.). At final harvest (70 d.a.s) the number of leaves per plant varied between 13 and 18. Two days prior to the first measurements damaged plants as well as the smallest and the largest plants per accession were removed after which 40 plants per accession were left. At this point the experiment was arranged in a randomised block design with four blocks. Each block contained ten plants per accession. The whole experiment was surrounded with border plants of the four accessions to enhance equal growth circumstances. At 31, 40, 49, 61 and 70 d.a.s. two plants per block of each accession (total 8 plants per accession per harvest date) were harvested. After each harvest the remaining plants within each block were rearranged.

Validation experiment

Plants from 'Jatilaba' (n=16), 'Bruinsma Wonder' (n=16), 'F_1 (Jatilaba x Bruinsma Wonder)' (n=16) and 25 F_3 lines of the cross between Jatilaba x Bruinsma Wonder (n=8 per line) were grown from December 2001 to March 2002 in a glasshouse with a light transmission of 63%. The average daily global radiation outside of the glasshouse was 3.3 MJ m^{-2} d^{-1}, ranging from 0.4 to 8.9 MJ m^{-2} d^{-1}. CO_2 was applied starting with 500 ppm CO_2 at 17 d.a.s to a maximum of 750 ppm CO_2 as from 35 d.a.s. Plant density was nine plants per m^2 during the whole experiment. At 68 d.a.s. five leaves per plant were harvested and measured. Measured leaves were 1) the first leaf of the side branches, 2) the second leaf from the top, 3) the second largest leaf (usually one of the upper leaves from the main stem), and 4 & 5) two leaves of intermediate leaf sizes.

Measurements

Length (L, in cm), width (W, in cm) and area (A, in cm^2) of single leaves were determined. Leaf length was defined as the distance from the top of the leaf to the branching point of the main vein and first lateral vein. Only leaves longer than 2.0 cm were used. Leaf width was measured as the widest region across the lamina perpendicular to the length. Actual area was measured with an area meter (Licor LI-3100). Leaf position was registered for each single leaf in the calibration experiment. Leaves at the main stem were numbered as leaf number A_1, A_2,…A_n (leafno_A) starting with the first true leaf. Leaves on each branch were numbered as leaf number B_{n+1}, B_{n+2},…, B_{n+m} (leafno_B). For each separate side branch, numbering was continued from the main stem. In calculations, leafno_B was set to zero for leaves on the main stem and leafno_A was set to zero for leaves on the side branches.

Calculations and statistics

Prior to calibration, the data set of the calibration experiment was randomly divided into two sets. The first set was used to calibrate the models, the second set for validation. The relation between area and length and width was determined using regression analysis on data of single leaves from the calibration set. Response variable "AREA" was first regressed on explanatory

variables L, L^2, W, W^2, L^2•W, W^2•L, L•W, $(L•W)^2$ and later also with the product of these terms multiplied with leafno_A and leafno_B. The effect of plant age (days after sowing, DAS) and accession were tested as factors in the regression analysis. Selection of the most accurate model for leaf area estimation containing multiple explanatory terms was done with an all subset regression analysis. In this method various regression models are tested with an increasing number of explanatory variables, starting with one. For each number of explanatory variables the best fitting models were given sorted by increasing adjusted R^2. Models with the highest adjusted R^2 in which all explanatory variables contributed significantly to the fit of the model were further analysed. Mean Square Error (MSE) and the value of the coefficients were calculated by multiple regression analysis and the final model was selected based on the combination of the highest R^2 and lowest MSE. An F-test was used to check if the model fit improved significantly by addition of these explanatory variables or factors (Sokal and Rohlf, 1981). Model A was the model with the highest number of explanatory variables or factors. RSS was the residual sum of squares, RMS the residual mean square and df was the number of degrees of freedom of the residual. F values were calculated using following equation:

$$F_{((df_B - df_A);df_A)} = \frac{(RSS_B - RSS_A)/(df_B - df_A)}{RMS_A}$$

Validation of the models was performed on the validation set of the calibration experiment and on data of the validation experiment. Linear regression analysis with calculated single leaf area as an explanatory variable and measured single leaf area as response variable were used to test various models. Both one way and two way ANOVA analysis was performed to compare values of measured and calculated total plant leaf area of the total data set of the calibration experiment. All tests used are standard procedures in Genstat 6.01 (Payne *et al.*, 2002).

Calculation of the total plant leaf area

Total plant leaf area was calculated as the sum of the areas of all single leaves per plant. To calculate the minimal number of leaves necessary to estimate total plant leaf area properly, area of non-measured leaves was estimated from leaves surrounding the non-measured leaf. Main stem and side branches were treated separately. When 75% or 25% of the leaves were considered, these were evenly distributed over the branches. In case of the 50% estimation, the odd numbered leaves were used. Area of the non-measured leaves was estimated as the average value of the nearest measured leaf above and below the non-measured leaf or as half of the area of the leaf below the missing leaf (in case of the last leaf on the branch). For the 10% estimation, one leaf at the central position on the main stem and one leaf per branch were used as a value for all leaves at that branch.

RESULTS

A total number of 1533 leaves from the four *Capsicum* accessions were measured for leaf area, length and width in the calibration experiment. Area of the leaves ranged from 0.73 to 136 cm^2, length from 2.0 to 18.5 cm and width from 0.6 to 10.5 cm. 'Bruinsma Wonder' and 'Nassau F_1' produced larger leaves than 'Jatilaba', F_1 '(Jatilaba x Bruinsma Wonder)' leaves were intermediate. Leaf shape also varied between the different accessions (Figure 2.1). 'Jatilaba' had narrow leaves (length:width ratio = 2.7) whereas 'Bruinsma Wonder' and 'Nassau F_1' had wider leaves (length:width ratio = 1.76 resp. 1.74). 'F_1 (Jatilaba x Bruinsma Wonder)' had an intermediate leaf shape (length:width ratio = 2.27).

Model calibration

To develop a method to estimate leaf area from length and width measurements independent of accession and leaf developmental stage, regression analysis was performed on a set of 766 leaves measured in the calibration experiment (calibration set). Area of single leaves could be predicted on basis of either length or width alone but predictions improved

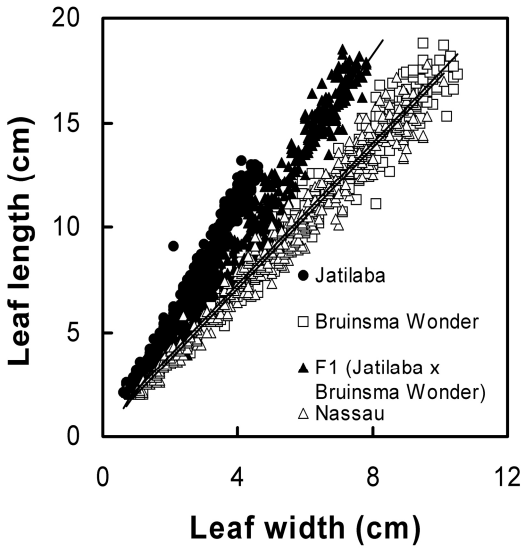


Figure 2.1: *Relation between leaf width (W) and leaf length (L) of single leaves from four measured Capsicum accessions measured in the calibration experiment. This figure includes the length and width of leaves of all ages and accessions. The lines represent linear regression lines for the different accessions: ● Jatilaba (L = 2.7•W; R^2 = 0.94), □ Bruinsma Wonder (L = 1.76•W; R^2 = 0.97), ▲ F_1 (Ja x BW) (L = 2.27•W; R^2 = 0.96) and ∆ Nassau (L = 1.74•W; R^2 = 0.97).*

when quadratic terms were added (Table 2.1). Further improvement was possible when the products of length and width (L•W or $(L•W)^2$) were used. None of the models in Table 2.1 was independent of accession and plant age. Furthermore, total plant leaf area was not predicted accurately for plants younger than 61 days using the model AREA = 0.690•L•W (Table 2.2).

To find a model to predict leaf area accurately for plants of all accessions and ages, the addition of L, L^2, W, W^2, L^2•W, W^2•L, $(L•W)^2$ to the model AREA = α•L•W was examined by regression analysis. The best fitting model (R^2 = 0.995; MSE = 5.2; P < 0.001) contained three variables and reads (Model 1):

$$AREA = 0.61906•L•W + 0.2060•W^2 – 0.5142•W \quad (1)$$

Table 2.1: *Coefficients (α, β) of the models used to estimate area (A) of single leaves from length (L) and width (W) measurements. Correlation coefficients (R^2) and mean squares errors (MSE) of the various models are also given. Data derived from a part of the calibration experiment (calibration set; 766 leaves) in which four* Capsicum *accessions were measured*

Model	α	β	R^2	MSE
$A = \alpha \cdot L$	4.35		0.734	263.9
$A = \alpha \cdot L + \beta \cdot L^2$	-0.415	0.376	0.92	79.5
$A = \alpha \cdot W$	8.78		0.822	176.5
$A = \alpha \cdot W + \beta \cdot W^2$	1.31	1.11	0.966	34.1
$A = \alpha \cdot (L \cdot W)$	0.69		0.993	6.8
$A = \alpha \cdot (L \cdot W) + \beta \cdot (L \cdot W)^2$	0.649	0.00037	0.994	5.8

Compared to the model AREA = 0.690•L•W, the explanatory power of Model 1 was significantly better ($P < 0.001$). Neither DAS nor accession contributed significantly to the fit of Model 1 ($P > 0.05$) showing that this model was independent of plant age and accession. When the Capsicum accessions were tested separately the same was found for plant age.

Subsequently, the relationship between leaf area and leaf position was evaluated. Regression analysis was performed with explanatory variables L, L^2, W, W^2, L^2•W, W^2•L, L•W, $(L \cdot W)^2$ and their products after multiplication with leaf position on the main stem (leafno_A) or leaf position on the side branches (leafno_B). This led to a model in which both leafno_A and leafno_B were represented (Model 2):

$$AREA = 0.5309 \cdot L \cdot W + 0.2399 \cdot W^2 - 0.2089 \cdot W + 0.007158 \cdot (L \cdot W \cdot leafno\_A) + 0.005475 \cdot (L \cdot W \cdot leafno\_B) \quad (2)$$

Addition of the leaf number terms to Model 1 increased the R^2 values from 0.995 to 0.996. MSE decreased from 5.2 to 3.8. The fit of Model 2 was significantly better than the fit of Model 1 ($P < 0.001$). Like Model 1, Model 2 was also independent of plant age and accession ($P > 0.05$).

Table 2.1: *Average total plant leaf area (in cm^2 ± s.e.) of eight plants per Capsicum accession for different days after sowing (d.a.s.). The percentage of difference between measured leaf area and calculated area are given for the model AREA=0.690•L•W, Model 1 and Model 2. Probability levels for differences between calculated and measured area are given per time point (P-value). Data of all measurements from the calibration experiment are included*

d.a.s.	Accession	Measured area (cm^2)	Deviation (%)		
			α•L•W	Model 1	Model 2
31	Jatilaba	6.7 ± 0.3	-11.5	-9.5	-5.2
	Bruinsma Wonder	11.3 ± 0.6	-6.9	-7	-4.5
	F_1 (Ja x BW)	15,0 ± 0.8	-11.9	-1.7	-0.7
	Nassau F_1	9.9 ± 0.4	-6.4	-8.6	-5.3
	P-value		***0.017***	***0.113***	***0.353***
40	Jatilaba	33.2 ± 1.3	-12.6	-1.5	-0.8
	Bruinsma Wonder	63.7 ± 2.6	-7.1	1	0.3
	F_1 (Ja x BW)	73.6 ± 4.4	-11.1	2.5	1.3
	Nassau F_1	64.4 ± 2.6	-6.6	0.1	0
	P-value		***0.02***	***0.805***	***0.904***
49	Jatilaba	95 ± 2.4	-10.5	-0.4	-0.5
	Bruinsma Wonder	229 ± 5.5	-4.1	1.7	0.5
	F_1 (Ja x BW)	228 ± 3.3	-10.2	4.5	3.4
	Nassau F_1	198 ± 12.4	-2.7	-0.5	-0.8
	P-value		***0.023***	***0.523***	***0.703***
61	Jatilaba	257 ± 6.0	-6.1	-2,0	-1,0
	Bruinsma Wonder	751 ± 14.8	0.7	0	-0.2
	F_1 (Ja x BW)	638 ± 13.8	-5.4	2.3	2.1
	Nassau F_1	598 ± 28.7	1.8	-1.8	-1.5
	P-value		***0.512***	***0.99***	***0.997***
70	Jatilaba	367 ± 10.3	-3.2	-3.9	-2.9
	Bruinsma Wonder	1154 ± 40.7	2	-0.4	0.2
	F_1 (Ja x BW)	907 ± 30.4	-3.4	0.8	1
	Nassau F_1	995 ± 31.8	3.9	-2.6	-2.1
	P-value		***0.661***	***0.675***	***0.833***

Model validation

The correlation between calculated area using either Model 1 or Model 2 and measured area was examined on the validation set derived from the calibration experiment (Figure 2.2). High correlations between calculated and measured leaf area were found for Model 1 (R^2 = 0.994; MSE = 5.4) and Model 2 (R^2 = 0.996; MSE = 4.0) when leaves of all four *Capsicum* accessions were pooled. The average ratio between calculated and measured area was 1.00 for both models. Averaged per accession, the ratio between calculated and measured area varied from 0.998 to 1.03 and from 0.94 to 1.02 for Model 1 and Model 2 respectively. Model 2 predicted leaf area better than Model 1: underestimation decreased from 2% to 1% and overestimation was reduced from 3% to 2%.

Model 1 was also validated on data of the validation experiment. In this experiment 'Jatilaba', 'Bruinsma Wonder', F_1 '(Jatilaba x Bruinsma Wonder)' and 25 F_3 lines (Jatilaba x Bruinsma Wonder) were measured for leaf length, width and area (leaf number was not recorded). Calculated area and measured area of all accessions pooled together were highly correlated (R^2 =

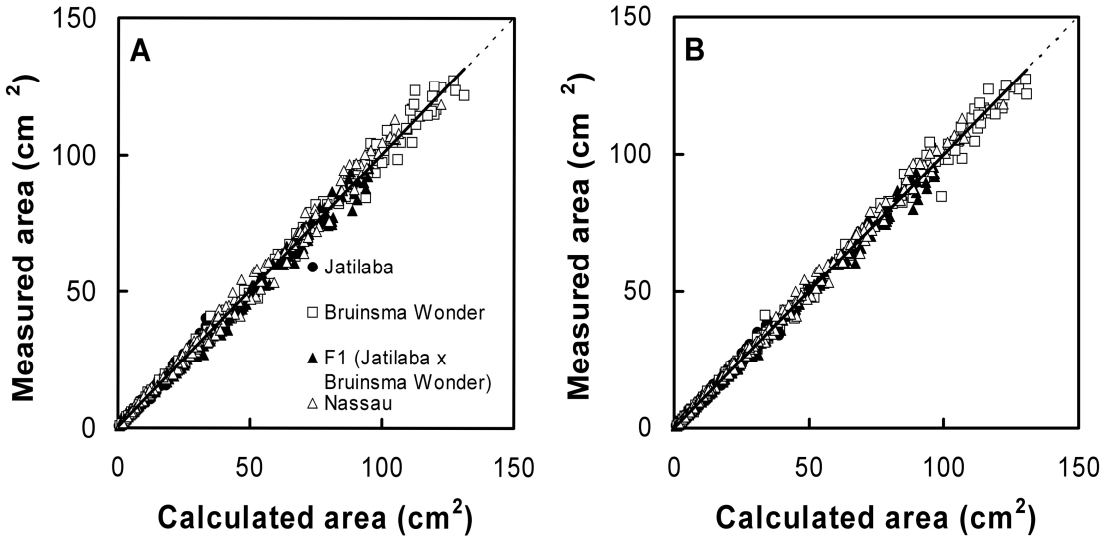


Figure 2.2: *Relation between calculated leaf area using (A) Model 1 (R^2 = 0.994; MSE = 5.4) or (B) Model 2 (R^2 = 0.996; MSE = 4.0) and measured area of single leaves from four Capsicum accessions. Data originate from the validation set of the calibration experiment. Solid lines represent the linear regression lines; dotted lines represent the 1:1 relationship. Symbols indicate the different accessions tested.*

0.992; Figure 2.3). The average ratio between calculated area and measured area was 0.96. 'Jatilaba' had the lowest R^2 value (R^2 = 0.990) and 'Bruinsma Wonder' the highest (R^2 = 0.997). The average ratio between calculated and measured area varied from 0.93 for 'Bruinsma Wonder' to 1.00 for 'Jatilaba'.

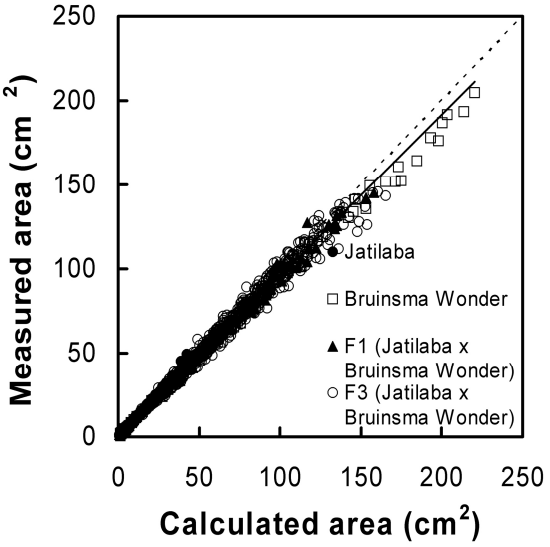


Figure 2.3: *Relation between calculated leaf area (using Model 1) and measured area of single leaves from three Capsicum accessions and 25 F_3 lines measured in the validation experiment. The solid line represents the lines regression line (R^2 = 0.992; slope = 0.96; P < 0.001); the dotted line represents the 1:1 relationship. Symbols indicate the different accessions tested.*

Total plant leaf area

To examine the consequences of deviations in the prediction of single leaf area for total plant leaf area, total plant leaf area was calculated using Model 1 and 2. No significant difference between measured and calculated total leaf area were found (Table 2.2). Model 1 and 2 resulted in a considerable improvement of total plant leaf area estimation compared to the model Area = 0.690•L•W.

Estimates of total plant leaf area were also made in cases where leaf area was calculated with Model 2 on length and width of 100%, 75%, 50%, 25% or 10% of the leaves (area of non-measured leaves was estimated; Table

2.3). As long as 25% or more of the leaves was measured, no significant differences were found between measured and calculated total plant leaf area ($P > 0.05$). This result shows that with only 25% of the leaves measured, an accurate total plant leaf area can be calculated. When 10% of the leaves were measured, significant differences between measured and calculated area of the total plant were found. Similar results were obtained with Model 1.

Table 2.3: *Measured area (in cm^2 ± s.e.) and percentage of difference between measured and calculated leaf area (Model 2) per plant when area was calculated on 100, 75, 50, 25 and 10% of leaves per individual plant. Data from the measurements at 40, 49, 61 and 70 d.a.s were used (32 plants per accession). Differences between calculated and measured area were not significant at P > 0.05 (n.s.) or significant at P < 0.001(*). Data derived from the calibration experiment*

	Measured	Difference between measured and calculated area (%)				
Accession	**Area (cm^2)**	**100%**	**75%**	**50%**	**25%**	**10%[1)]**
Jatilaba	188 ± 24	-1.2 n.s.	-0.3 n.s.	-3.2 n.s.	-2.4 n.s.	28 *
Bruinsma Wonder	549 ± 78	0.4 n.s.	1.0 n.s.	-1.7 n.s.	-1.1 n.s.	24*
F_1 (Ja X BW)	461 ± 60	2.0 n.s.	2.5 n.s.	0.1 n.s.	-1.0 n.s.	28*
Nassau	464 ± 66	-0.9 n.s.	2.0 n.s.	-3.2 n.s.	-6.4 n.s.	13*

[1)] At 40 d.a.s. the number of leaves per plant varied from 5 to 7. Therefore the percentage of leaves measured was between 14 and 20%.

DISCUSSION

In this paper we describe how leaf area can be estimated from simple non-destructive measurements in *Capsicum*. Several models have been described for estimating leaf area from measurements of length and width in numerous crops, but only little information was available for *Capsicum*. The most simple model found in the current study was Area = 0.690•L•W (Table 2.1). This model is dependent on both plant age and accession and does not predict total plant leaf area accurately for younger plants (Table 2.2). Models for *Capsicum*

with the same structure published before also show age-related variation (Shivashankar *et al.*, 1986) and differences between accessions (Ray and Singh, 1989). Kläring *et al.* (1996) stated that leaf area in *Capsicum* could be predicted based upon a model with L^2 alone and Bakker (1989) stated that this could be done based on width alone. In our study however, simple models containing other variables give a considerably better prediction, especially when the product of length and width is included. We therefore conclude that both length and width measurements are necessary to estimate leaf area accurately.

Models independent of accession and plant age were obtained when leaf width terms (W, W^2) alone (Model 1) or in combination with leaf position terms (L•W•leafno; Model 2) were added to the term L•W (Figure 2.2). During the whole vegetative growth phase, these models can be used to estimate total plant leaf area properly (Table 2.2) as long as more than 25% of the leaves per plant is measured (Table 2.3).

When individual leaves grow older, they become more rounded resulting in a higher ratio between leaf area and L•W, as was also reviewed by Marshall (1968). A model only containing L•W cannot compensate for the age dependent variation in this ratio, while models with the additional leaf width terms (W, W^2) can: the area of small leaves is negatively influenced by these terms whereas larger leaves are positively influenced. Furthermore, leaf shape is dependent on leaf position as indicated by the leaf position terms (Model 2) and as is known from literature (Esau, 1965; Wardlaw, 1968). Addition of separate leaf position terms for main stem and side branches gives a significantly better relation to single leaf area than the addition of a general leaf position term for all leaves. The coefficients for the main stem and side branches have significantly different values suggesting that leaves on the main stem have a different shape than leaves on the side branches. Similar results were found by Verwijst and Wen (1996)in *Salix*. Despite the developmental importance, the practical value of the leaf position terms is limited. Validation of Model 2 has only been performed at plants with 9 to 13 leaves below the branching point. For accessions with a different number of leaves before branching, the leaf position terms might decrease the accuracy of the model.

The model containing only the leaf width terms (W, W^2) in addition to L•W (Model 1) was used to estimate leaf area accurately in a set of 200 genetically unique F_3 *Capsicum* plants, 'Jatilaba' and 'Bruinsma Wonder' and their F_1 (Figure 2.3) grown under considerable different conditions than plants in the calibration experiment. The maximum leaf area in the validation experiment was 205 cm^2 whereas this was 136 cm^2 in the calibration experiment. The increase in individual leaf size can be the result of the higher light intensities during this experiment, as was also found in *Capsicum* by Nilwik (1981b) and Heuvelink and Marcelis (1996). In *Aglaonema commutatum* both leaf area and length:width ratio were reduced upon lower radiation (Di Benedetto and Cogliatti, 1990). Furthermore, elevated CO_2 can also promote the individual leaf size (Ferris *et al.*, 2001; Taylor *et al.*, 2001) and altered leaf shape (Taylor *et al.*, 2003; Thomas and Bazzaz, 1996). Although the environmental conditions in the validation experiment could have influenced both leaf size and shape, the ratio between calculated and measured area for all accessions together is still close to unity (mean 0.96, range from 0.93 to 1.00). This shows Model 1 is a robust model that can be used for genetically unique plants and plants grown under different environmental conditions. Therefore, this Model 1 is of great value for physiological studies and breeding purposes and the addition of the leaf position terms can be seen as a refinement of this model.

Chapter 3

Variation in relative growth rate and growth traits in wild and cultivated Capsicum accessions grown under different temperatures

Erik A.M. de Swart, Leo F.M. Marcelis, Roeland E. Voorrips (2006)

Journal of Horticultural Science & Biotechnology 81: 1029-1037

ABSTRACT

Differences in environmental conditions are known to influence plant growth and growth related traits. The aim of this study was to identify the variation in relative growth rate (RGR), and its underlying physiological and morphological traits, in a group of ten wild and cultivated *Capsicum* accessions of four *Capsicum* species at two contrasting temperatures. Therefore, a growth analysis was performed at two temperature regimes (21.1°/18.7°C and 17.3°/14.7°C; day/night). Variation in RGR, growth related traits and their mutual relationships were analysed at the start of branching. RGR at branching proved to be a reliable predictor for dry mass development pre-anthesis in *Capsicum*. The reduction in RGR and growth-related traits in response to lowered temperatures varied between these *Capsicum* accessions; some being better adapted to low temperatures than others. The variation in the reduction of RGR under lowered temperatures was due to changes in both net assimilation rate (NAR) and leaf area ratio (LAR); dry mass allocation to the leaves (leaf mass fraction) was not influenced by temperature. Specific leaf area (SLA) was correlated with RGR under standard temperatures, but not under lowered temperatures. At both temperatures, NAR was the most important factor to explain variations in RGR between the different *Capsicum* accessions. From this study, and the work of others, it was concluded that NAR was the most important factor to explain variations in RGR in plants of the same genus or species, and even between closely-related plant species.

INTRODUCTION

The genus *Capsicum* consists of more than 20 wild species and five domesticated species (Bosland, 1994; Poulos, 1993) that originate from South and Central America (Bosland and Votava, 2000; Heiser and Smith, 1953). *Capsicum* species occur in a wide range of different habitats with average day temperatures between 7° - 29°C, annual precipitation between 0.3 - 4.6 m, and a soil pH between 4.3 - 8.7 (Simon *et al.*, 1984). Some species, for example *C. chinense*, are confined to lowland areas, whereas *C. pubescens* is found only in highlands of South and Central America. *C. annuum* is found in a wide range of altitudes (Heiser, 1964). In general, *Capsicum* species are cold-sensitive and grow best in well-drained, sandy or silt-loam soil (Simon *et al.*, 1984). This wide range of growth habitats makes *Capsicum* spp. interesting subjects for ecophysiological studies.

Differences in environmental conditions such as temperature, nutrient availability and altitude have an influence on plant growth. Grime and Hunt (1975) were the first to show that different species originating from unfavourable environments tend to have a low inherent relative growth rate [RGR; increase in dry mass (DM) per unit biomass per unit of time] under non-limiting growth conditions, whereas plants that occur naturally in favourable habitats have an inherently high RGR. Grime and Hunt (1975) suggested that this variation in RGR reflected an adaptation to stress conditions; Lambers and Poorter (1992) argued further that it was not RGR itself, but one of the growth-related traits linked with RGR that was the target for selection.

RGR is the product of two growth related traits: net assimilation rate (NAR; increase in DM per unit leaf area and per unit time) and leaf area ratio (LAR; the leaf area per unit of total plant DM). NAR is a physiological parameter and is the net result of carbon gain due to photosynthesis and carbon losses, due mainly to respiration. LAR is a morphological parameter and is the product of specific leaf area (SLA; leaf area per unit leaf DM) and leaf mass fraction (LMF; the fraction of total plant DM allocated to the leaves; Evans, 1972). SLA itself depends on the chemical composition (Garnier and Laurent, 1994) and anatomy of leaves (Van Arendonk and Poorter, 1994).

In many studies on the relationship between RGR and growth related traits, different species are grown at one, more-or-less optimal, temperature. Under these conditions, LAR and, more specifically, SLA, are the most important factors to explain the inherent variation in RGR between species, whereas differences in NAR are thought to be of secondary importance (reviewed by Poorter, 1989a; Poorter and Van Der Werf, 1998). On the other hand, NAR seems to be of greater or at least equal importance as LAR in determining differences in RGR between more closely-related accessions, belonging to the same genus or species (Biere, 1996; Van der Ploeg *et al.*, 2005; Verhoeven *et al.*, 2004; Villar *et al.*, 1998).

The response of NAR to temperature can be described as a curve with an optimum close to the optimal growth temperature (i.e., the temperature where the highest RGR is reached; Hunt and Halligan, 1981), or a temperature comparable to the natural growth temperature (Bednarz and Van Iersel, 2001; Soldati *et al.*, 1999; Van Iersel and Lindstrom, 1999). In studies in which the temperature range was relatively small, an effect of temperature on NAR was often not detected, probably because the different temperatures were close to the optimum temperature (Bruhn *et al.*, 2000; Stirling *et al.*, 1998). The relative importance of growth related traits on RGR, between different species, also depends on temperature (Loveys *et al.*, 2002).

For the genus *Capsicum*, information on the influence of temperature on the relationship between RGR and growth related traits has been limited to studies using only one *Capsicum* accession (Nilwik, 1980a, b, 1981a, b). More information is available on the influence of light intensity (Bruggink, 1987; Bruggink and Heuvelink, 1987) and salinity (Hegde, 1987; Villa-Castorena *et al.*, 2003) on RGR and its components. In the current study, the effect of temperature on RGR and its underlying growth traits was examined in a group of ten wild and cultivated *Capsicum* accessions representing four *Capsicum* species, and originating from four continents.

Sweet pepper, grown in glasshouses, requires a high growth temperature and hence a high energy input. Except for technical measures (Bot, 2001) and dynamic climate control (using the ability of a crop to compensate for temperature changes within a limited period; Ottosen *et al.*, 2003), growing pepper plants at a lower temperature is one of the options to

reduce energy consumption. Breeding for low temperature tolerance will allow growers to grow pepper at lower temperature and reduce energy consumption. Therefore, the aim of this study was to identify *Capsicum* accessions that showed only a small decrease in RGR at lower temperatures, and to identify the growth-related traits that contribute to this adaptation. Wild *Capsicum* accessions were chosen based on average daily temperatures in their natural habitats as this was expected to be related to their optimal growth temperature. To discriminate between treatment and ontogenetic effects, RGR and growth traits were compared in plants at the same developmental stage.

MATERIALS AND METHODS

Plant material

Ten *Capsicum* accessions from different habitats were collected (Table 3.1). *C. annuum* 'Bruinsma Wonder', 'Jatilaba', F_1 '(Jatilaba x Bruinsma Wonder)', and *C. chinense* 'PI 159233' were included because they have been the subjects of earlier studies (De Swart *et al.*, 2004a). The wild accessions were chosen based on the average day temperature in their natural habitat compared to standard Dutch glasshouse conditions.

Plant cultivation

Plants were grown in a glasshouse in Wageningen, The Netherlands (latitude 52 °N) from September 2003 to January 2004. The glasshouse had a light transmission of approxi. 60%. The average daily global radiation outside the glasshouse was 3.4 MJ m^{-2} d^{-1}, ranging from 0.3 - 11.1 MJ m^{-2} d^{-1}. Seeds were sown in rockwool plug trays (25 mm x 25 mm x 40 mm $plug^{-1}$), moistened with water and covered with fine vermiculite. Germination occurred at a day/night temperature of 21°/19°C. Fourteen days after sowing (d.a.s.), seedlings were transplanted into rockwool cubes (10 cm x 10 cm x 10 cm) and, from that time, drip-irrigated with nutrient solution (EC = 2.1 dS m^{-1}; pH = 5-6) containing 0.5 mM NH_4, 6.75 mM K, 5.0 mM Ca, 1.5 mM Mg, 15.5 mM NO_3, 1.75 mM SO_4, 1.25 mM P, 15 µM Fe, 10 µM Mn, 5 µM Zn, 30 µM B, 0.75 µM Cu and 0.5 µM Mo.

Table 3.1: *List of Capsicum accessions studied and additional information about their original habitat*

Accession	Origin	Population	Temp[a] (°C)	Altitude (m)
C. annuum 'Bruinsma Wonder' [1]	NL	OP cultivar	21	0
C. annuum 'Jatilaba' [2]	Indonesia	OP cultivar	27	0-300
C. chinense 'PI 159233' [1]	USA	OP cultivar	*b	*b
C. annuum 'PI 585238' [3]	Ecuador	Wild	17	1700
C. pubescens 'PI 585265' [3]	Ecuador	Wild	12	2780
C. baccatum 'PI 585248' [3]	Ecuador	Wild	12	3000
C. baccatum 'PI 585241' [3]	Ecuador	Wild	11	3063
C. chinense 'PI 543184' [3]	Bolivia	Wild	22	630
C. annuum x C. annuum F_1 'Jatilaba x Bruinsma Wonder' [4]	NL	F_1	21	0
C. annuum x C. annuum F_1 'PI 585238 x Bruinsma Wonder'[4]	NL	F_1	*c	0

[a] *Average daily temperature in the natural habitat or normal cultivation.*

[b] *Exact origin and corresponding average daily temperature unknown.*

[c] *No data available*

[1] *Seeds were obtained from the CGN (Wageningen, The Netherlands).*

[2] *Seeds were obtained from the RIV (Lembang, Indonesia).*

[3] *Seeds were obtained from The National Germplasm System (USA).*

[4] *F_1's were produced at Plant Research International (Wageningen, The Netherlands).*

Capsicum accessions used in this experiment developed a main stem that ended with one or two flowers, and branched into two or three side branches. At each first or higher order branch, one leaf developed and the branch terminated in a flower and divided into two or three higher-order branches. In this experiment, two first order branches were retained. Subsequently, the largest of each higher-order branch was retained, while the smallest branch was removed above the first leaf.

The different temperature treatments started 14 d.a.s. (day/night = 16 h/8 h). Plants were grown in four glasshouse compartments that formed an E-W linear array. Two compartments had actual average day/night temperatures of 21.1°/18.6°C and 21.2°/18.7°C, respectively (standard temperatures): The

other two glasshouse compartments had average day/night temperatures of 17.3°/14.7°C and 17.2°/14.7°C, respectively (lowered temperatures). The 24-h average temperatures of the different compartments were 20.3°, 20.5°, 16.5° and 16.4°C. The initial values for relative air humidity at the start of the temperature treatments varied between 41% - 45% (standard temperatures) and 52% - 55% (lowered temperatures). During the next eight days, values for relative air humidity increased and became stable at values of 66% - 69% for both temperature treatments. Supplementary light (25 µmol m^{-2} s^{-1} PAR) was provided by HPI-T lamps between 0600 - 2200 h, when the global radiation outside the glasshouse was lower than 100 J m^{-2} s^{-1}, following the same day-night scheme as the temperature.

Experimental design

The experiment was arranged in a split-plot design with four whole plots (compartments), and 40 sub-plots within each compartment (i.e. four sub-plots per accession). The whole experiment was surrounded with guard plants of each of the ten accessions. Each sub-plot consisted of two rows of plants, each with one guard plant at both ends of each row. The distance between the rows was 40 cm. A sub-plot contained ten experimental plants of which the largest and smallest plant were removed prior to the second harvest to minimise plant-to-plant variation (Poorter, 1989b). At 15, 21, 28, 35, 42, 49 and 63 d.a.s., one randomly chosen plant per sub-plot was harvested. After each harvest, the remaining plants within each sub-plot were rearranged, such that the distance between the plants in a row increased from 11.5 cm to 40 cm during the experiment. An additional harvest was performed for the lowered temperature treatments at 98 d.a.s. For each harvest, a total of eight plants per accession were measured for each temperature treatment.

Measurements

At each harvest, plant height was measured as well as the height of the branching point and the number of leaves above and below the branching point. Subsequently, plants were separated into two fractions: leaf blades and the remaining above-ground fraction (consisting of the stems and petioles). Fresh masses of each fraction, as well as the areas of the leaf blades using a

Licor LI-3100 leaf area meter (Licor, Lincoln, NE, USA) were determined. Dry masses (DM) were determined on oven-dried (48 h at 70 °C) material. Time of branching was also recorded.

Calculations

Relative growth rate and NAR were calculated for each time interval (eight plants per time point) as described by Hunt *et al.* (2002): LAR, SLA and LMF were calculated for each single plant as described by Hunt (1982). The developmental scale, expressed as % NRLV, was calculated as the total number of leaves per single plant as a % of the average number of leaves at the moment of branching of the corresponding accession in the same temperature treatment:

% NRLV = (number of leaves / number of leaves at branching) • 100

where the average %NRLV at branching was, by definition, 100%.

In order to eliminate changes in growth rate due to differences in ontogenetic stage, the 75 – 120% developmental interval was chosen to make comparisons between the growth rates of the different *Capsicum* accessions at the two temperatures, and to correlate RGR with NAR, LAR, SLA and LMF. First, RGR and NAR values, calculated per time interval, were placed on the developmental scale. This placement was based on the average number of leaves of all plants per accession in a time interval. Subsequently, the 75% and 120% NRLV border values for RGR and NAR were determined by interpolation of the RGR and NAR values flanking the borders. The averages of the values (at 75% and 120% NRLV) of RGR and NAR were used for further analysis. Values of LAR, SLA and LMF for each accession were the average values of these traits for all plants used to calculate the RGR and NAR over the 75 – 120% developmental interval.

Relationships between RGR and other plant parameters were analysed by linear regression of mean accession values. Analysis of Variance (ANOVA) was used to analyse the main effects and interactions of accession and temperature. Growth response coefficients (GRC; Poorter and Van Der Werf, 1998) were used as a measure for the proportion in which a particular growth

parameter explained the RGR differences between accessions. GRC has a value of 1 if the change in a particular trait is fully proportional to the increase in RGR: A GRC value of 0 indicates that the difference in RGR was not accompanied by any systematic difference in this trait. GRC values below 0 and above 1 may also occur, for example if a higher RGR is accompanied by both a lower NAR and a more than proportionally higher LAR. GRC values were estimated as the slopes of linear regressions with ln(NAR), ln(LAR), ln(SLA) or ln(LMF) values as response variable and ln(RGR) as the explanatory variable. All statistical analyses were performed using the Genstat 6.0. statistical package (Payne *et al.*, 2002).

RESULTS

Growth and growth-related traits of plants of ten different wild and cultivated *Capsicum* accessions were analysed at two contrasting day/night temperature regimes (21.1°/18.7°C and 17.3°/14.7°C). One day after the start of the temperature treatment (15 d.a.s.), the DMs of plants of the same accession were not significantly different between temperature treatments ($P > 0.05$). From 21 d.a.s., plants grown under standard temperatures had significantly higher DM values than plants grown under lowered temperatures (Figure 3.1). At the final harvest of plants grown under standard temperatures (63 d.a.s.), DM varied from 1.30 g for *C. chinense* 'PI 543184' to 7.42 g for F_1 '(PI 585238 x Bruinsma Wonder)'. Under lowered temperatures, values for DM at this harvest date varied from 0.23 g for *C. chinense* 'PI 543184' (1.33 g at 98 d.a.s.) to 1.69 g for F_1 '(PI 585238 x Bruinsma Wonder)' (11.40 g at 98 d.a.s.). The DM of the different accessions at the final harvest of plants grown under standard temperatures was positively correlated to that at lowered temperatures ($R^2 = 0.71$; $P < 0.001$).

Growth rates

RGR values for each *Capsicum* accession varied during development for both temperature treatments. In general, the maximum RGR (RGR_{max}) was reached in the time interval when the first true leaves appeared and, from that moment

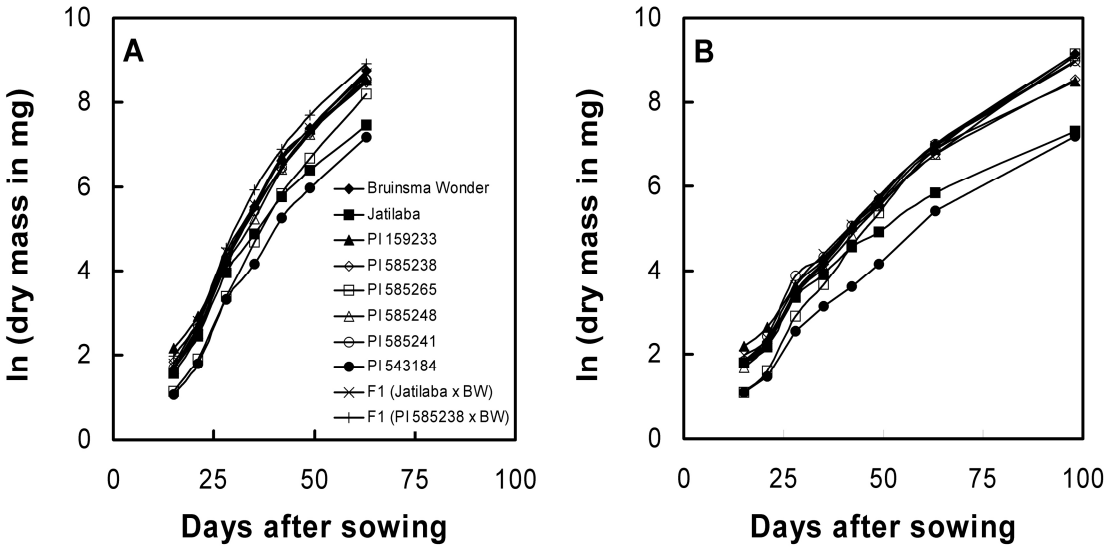


Figure 3.1: *Total dry mass (ln (dry mass)) over time (days after sowing) in ten Capsicum accessions grown under (A) standard (21.1°/18.7°C) or (B) lowered (17.3°/14.7°C) temperatures.*

onwards, decreased during plant development. RGR_{max} values varied from 203 mg g^{-1} d^{-1} for *C. chinense* 'PI 159233' to 251 mg g^{-1} d^{-1} for *C. annuum* 'PI 585238' at standard temperatures, and from 142 mg g^{-1} d^{-1} for *C. chinense* 'PI 159233' to 186 mg g^{-1} d^{-1} for *C. pubescens* 'PI 585265' at lowered temperatures. NAR, LAR, SLA and LMF changed during development and eventually became more-or-less constant (data not shown). Because of these ontogenetic effects plants had to be compared at the same developmental stage rather than at the same number of d.a.s.

The onset of branching, when the generative phase starts, is a clear reference point during the physiological development of *Capsicum* plants. Therefore, a developmental scale was created based on the number of leaves relative to the number of leaves at the onset of branching. Consequently, the developmental stage at branching was 100%. Values for RGR, NAR, LAR, SLA and LMF were based on the 75% - 120% developmental interval.

The RGR of plants grown under standard temperatures in this developmental interval varied from 90 mg g^{-1} d^{-1} for *C. annuum* 'Jatilaba' to 150 mg g^{-1} d^{-1} for F_1 '(PI 585238 x Bruinsma Wonder)': Under lowered

temperatures, the RGR varied between 51 mg g^{-1} d^{-1} for 'Jatilaba' to 107 mg g^{-1} d^{-1} for *C. pubescens* 'PI 585265'. RGR was positively correlated with DM at final harvest under standard (R^2 = 0.57; P < 0.001) and lowered (R^2 = 0.57; P = 0.007) temperatures. No significant correlations (P > 0.10) were found between RGR_{max} and RGR at the time of branching, or between RGR_{max} and DM at final harvest.

Growth related traits

Under standard temperatures, a positive correlation was found between RGR and NAR (R^2 = 0.44; P = 0.02): The same trend (R^2 = 0.31; P = 0.06) was found when plants were grown under lowered temperatures (Figure 3.2A). LAR showed a quite different pattern, as it was not significantly correlated with RGR under standard or lowered temperatures (Figure 3.2B). LAR is a trait that depends on leaf morphology (SLA) and DM allocation to the leaves (LMF). Under standard temperatures, SLA correlated significantly (R^2 = 0.43; P = 0.03) with RGR, whereas this correlation was not significant under lowered temperatures (Figure 3.2C). The other component of LAR, LMF, was not significantly correlated with RGR (Figure 3.2D).

NAR and LAR were negatively correlated under both temperature regimes (Figure 3.3). The correlation between LAR and LMF was smaller under lowered temperatures. SLA, on the other hand, was only significantly correlated with LAR under standard temperatures.

Table 3.2: *Quantification of the relative importance of traits[*] underlying relative growth rate (RGR) to explain differences in the RGR of ten Capsicum accessions at two temperatures using Growth Response Coefficients (GRC)*

	Growth Response Coefficient	
Growth related trait	Standard temp	Lowered temp
NAR	0.73	0.69
LAR	0.18	0.25
SLA	0.32	0.24
LMF	-0.14	-0.10

[*] *Net assimilation rate (NAR); leaf area ratio (LAR); specific leaf area (SLA); leaf mass fraction (LMF)*

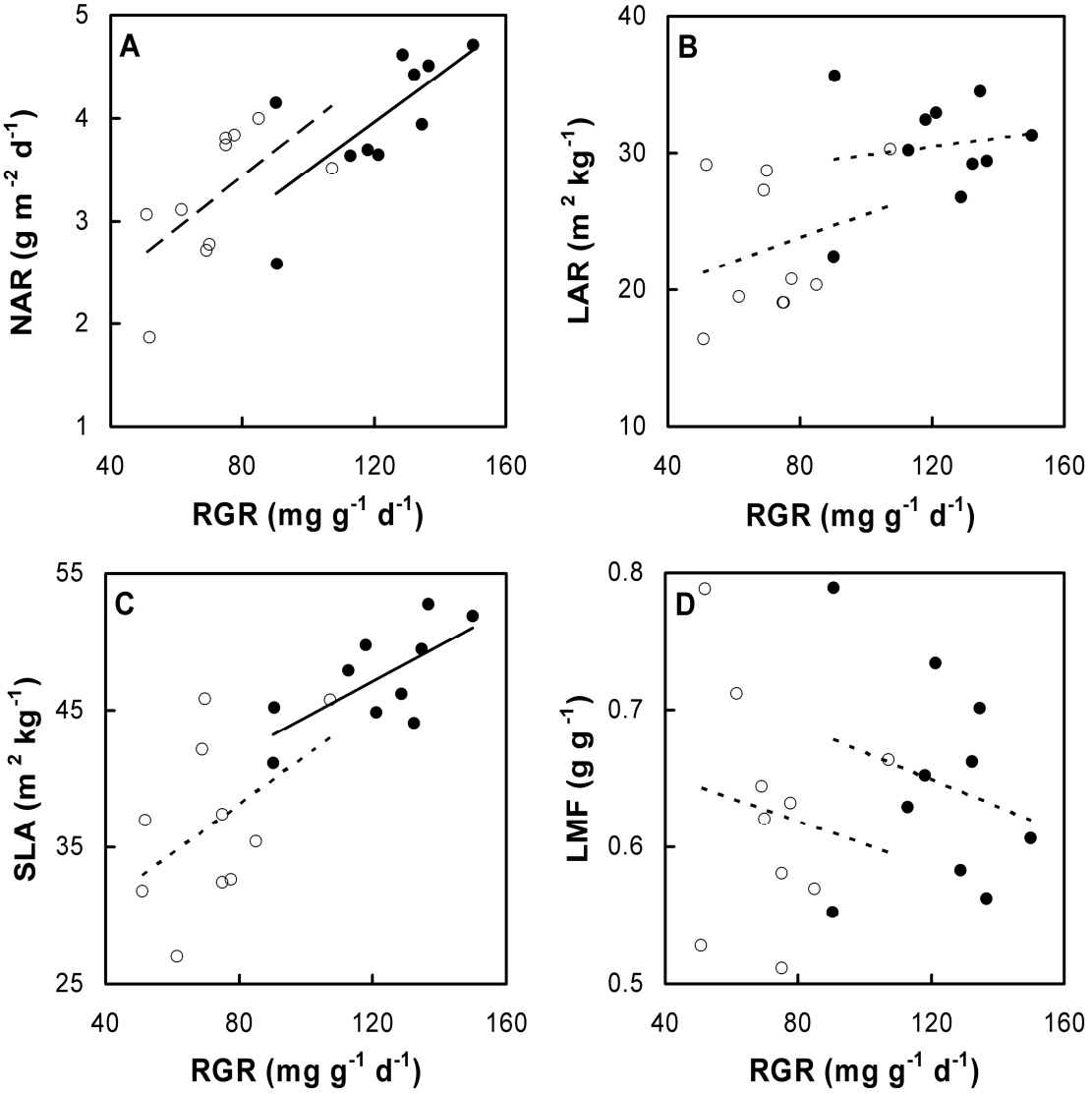


Figure 3.2: *Relation between mean values of relative growth rate and (A) net assimilation rate, (B) leaf area ratio, (C) specific leaf area and (D) leaf mass fraction for ten Capsicum accessions grown under (●) standard (21.1°/18.7°C) or (○) lowered (17.3°/14.7°C) temperatures. A solid line indicates a significant relationships at P < 0.05; Dashed lines indicate a significant relationship at 0.10 < P < 0.05; dotted lines indicate a non-significant relationship (P > 0.10).*

Growth response coefficients (GRC) were calculated to quantify the relative importance of traits underlying RGR in explaining the effect of temperature on differences in RGR of different accessions (Table 3.2). At both temperatures, GRC_{NAR} had the highest values, indicating that NAR was the most important trait explaining the difference in RGR under both temperatures. GRC_{LAR} was much lower than GRC_{NAR} under both temperatures indicating that LAR contributed less to variation in RGR than NAR. Under standard temperature, GRC_{SLA} was higher than GRC_{LAR} indicating that SLA explained a larger part of the variance in RGR than LAR. The relative importance of LMF was low under both temperature treatments.

Temperature effect on RGR and growth related traits

Under standard temperatures, RGR was significantly higher ($P < 0.001$) than under lowered temperatures. The RGR of plants grown under standard temperatures was positively correlated ($R^2 = 0.58$; $P = 0.007$) with the RGR of plants grown under lowered temperatures (Figure 3.4A). *C. pubescens* 'PI 585265' performed relatively better under lowered temperatures than the other accessions tested.

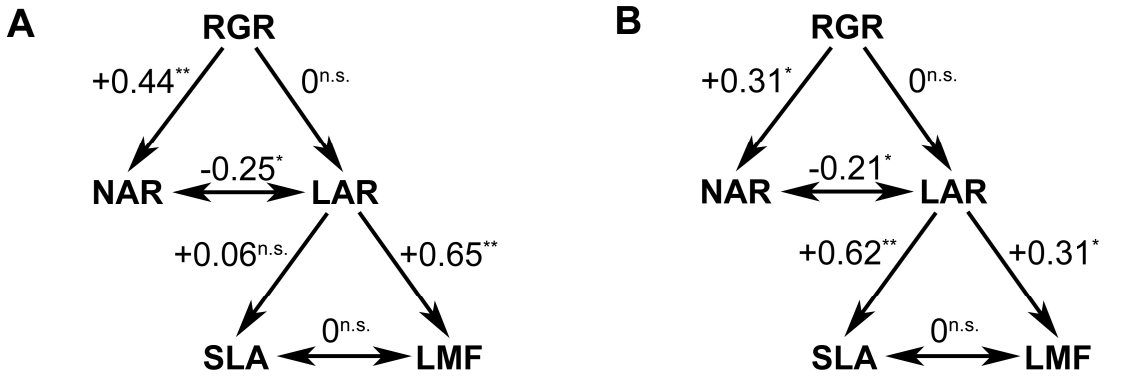


Figure 3.3: *Correlation coefficients (R^2) between RGR and various growth-related traits of Capsicum plants grown under (A) standard (21.1°/18.7°C) or (B) lowered (17.3°/14.7°C) temperatures. Signs (+ or -) indicate positive or negative correlations. Significance is indicated as ** (P < 0.05), * (0.10 < P< 0.05) or n.s. (P > 0.01).*

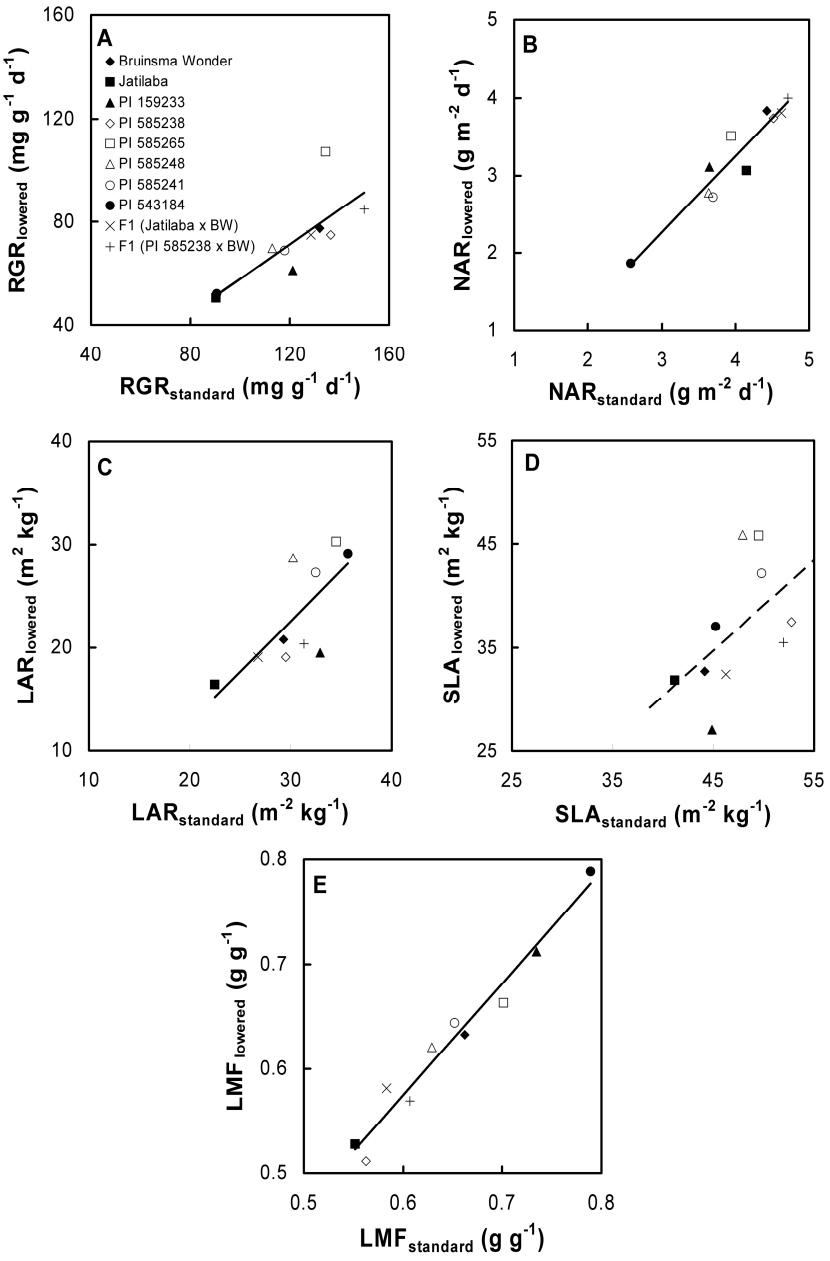


Figure. 3.4: *Relationships between mean values of (A) relative growth rate, (B) net assimilation rate, (C) leaf area ratio, (D) specific leaf area and (E) leaf mass fraction for ten* Capsicum *accessions grown under standard (21.1°/18.7°C) or lowered (17.3°/14.7°C) temperatures. A solid line indicates a significant relationship at P < 0.05; dashed lines indicate a non-significant relationship (P > 0.10)*

Values for NAR, LAR and SLA were significantly lower under lowered temperatures ($P < 0.001$), except for *C. baccatum* 'PI 585248'. In this accession, neither LAR nor SLA decreased significantly as a result of lowered temperatures. LMF was hardly affected by temperature in all accessions. Linear regression analysis showed (Figure 3.4B, C, E) that the NAR ($R^2 = 0.90$; $P < 0.005$), LAR ($R^2 = 0.48$; $P = 0.016$), and LMF ($R^2 = 0.96$; $P < 0.001$) of plants grown under standard temperatures were positively correlated to those at lowered temperatures: SLA values (Figure 3.4D) under both temperature treatments were not significantly correlated with each other ($P = 0.12$).

Interaction between temperatures and accessions

C. pubescens 'PI 585265' performed relatively better under lowered temperatures than the other accessions tested. The reduction in RGR between the temperature treatments was only 20% for *C. pubescens* 'PI 585265', whereas the average reduction for all accessions together was 41%.

The correlation between RGR at both temperatures increased from 0.58 to 0.71 ($P < 0.001$) when *C. pubescens* 'PI 585265' was removed. Both NAR and LAR (Figure 3.4B, C) contributed to the relative high growth rate of this accession under lowered temperatures since the decreases in NAR and LAR between the temperature treatments was relatively small (11% and 12%, respectively). All other accession showed larger decreases in NAR and LAR. With respect to LAR, only *C. baccatum* 'PI 585248' showed a smaller relative decrease between the two temperature treatments (5%) than *C. pubescens* 'PI 585265', but this was accompanied by a larger reduction in NAR (24%).

DISCUSSION

Under both temperature treatments, RGR_{max} was reached during the time interval in which the first true leaves appeared. Hanley *et al.* (2004) identified RGR_{max} as a reliable marker for the end of the seedling phase. Although RGR_{max} is an important (eco)physiological attribute (Garnier, 1991; Hanley *et al.*, 2004), it could not be used as a predictor for further plant development,

since it was not correlated to growth rate or DM during later plant development. After the appearance of the first true leaves, RGR and growth related traits gradually decreased, as was observed in *Capsicum* (Bruggink and Heuvelink, 1987; Hegde, 1987; Nilwik, 1981b; Pérez-Grajales *et al.*, 2004). To compensate for differences in developmental rate, RGR and growth related traits were compared at the same ontogenetic stage, the start of the generative phase. Values of RGR for both temperature treatments at that stage were positively correlated with final DM. Si and Thurling (2001a) also found a positive correlation between pre-anthesis RGR and DM at anthesis in *Brassica* under different temperatures. Therefore this RGR value is a reliable predictor for DM development in *Capsicum*.

The variation in RGR reduction due to lowered temperatures showed that some *Capsicum* accessions were better adapted to lowered temperatures than others (Figure 3.4A). This indicates that within *Capsicum* variation for low temperature tolerance is available for breeding varieties requiring less energy input. For breeding purposes, RGR is a much more informative trait than DM: RGR describes the DM development in time rather than DM at one single moment in time.

Growth related traits

The results of our study demonstrate that NAR is the most important growth related trait in explaining differences in RGR between accessions within the genus *Capsicum* (Figure 3.2A; Table 3.2). Four *Capsicum* species were analysed in our study. The taxonomy of *Capsicum* species, however, is a subject of ongoing discussion (Eshbaugh, 1980, 1993; Pickersgill, 1988; Smith *et al.*, 1987). Smith and Heiser (1957) showed that *C. baccatum* and *C. pubescens* are certainly two separate species. The status of *C. annuum* and *C. chinense* as distinct species, both belonging to the *C. annuum* complex, is questionable since they are closely related (Pickersgill, 1988).

Within accessions of the *C. annuum* complex, we found the same relations as across all *Capsicum* accessions; NAR was the most important factor to explain the variation in RGR (data not shown). Other studies between species belonging to the same genus (Atkin *et al.*, 1996; Van der Ploeg *et al.*, 2005; Villar *et al.*, 1998), or between accessions of the same species (Biere,

1996; Si and Thurling, 2001a; Verhoeven *et al.*, 2004), also demonstrated that NAR was of more, or at least equal importance as LAR in determining differences in RGR. Similar results were also found in comparative studies between closely-related grass species (Garnier, 1992; Reiser *et al.*, 2002). On the other hand, Poorter, (1989a) and Poorter and Van Der Werf (1998) concluded that LAR, and more specifically SLA, were the most important factors in explaining inherent variation in RGR in different species. Considering our own results, and the work of others, we conclude as a general rule that within plant genera, or even within species and between closely-related plant species, NAR is the most important factor to explain variation in RGR.

No correlation was found between RGR and LAR (Figure 3.2B). Moreover, GRC analysis showed that the relative importance of LAR was low compared to that of NAR (Table 2.2). LMF, one of the components of LAR, was not correlated with RGR. However, SLA, the other component of LAR was positively correlated with RGR under standard temperatures (Figure 3.2C). This relation was not found under lowered temperatures. In their study of 16 different plant species (trees, shrubs, grasses and forbs), Loveys *et al.* (2002) found that under moderate or high temperatures, SLA was the important factor affecting RGR whereas under lowered temperature, NAR became the most important determinant of RGR. Although they found a correlation between SLA and RGR, no evidence was presented for a correlation between RGR and LAR. In our opinion, SLA can only be a determinant of RGR when a correlation between RGR and LAR also exists.

Contribution to differences in RGR

Both NAR and LAR play an important role in the influence of temperature on RGR. *C. pubescens* 'PI 585265', the accession with the smallest decrease in RGR, exhibited the smallest decrease in NAR and the second smallest reduction in LAR (Figure 3.4A-C). *C. baccatum* 'PI 585248', with the smallest decrease in LAR, but an average reduction in NAR showed the second smallest decrease in RGR. In other accessions, the reduction in RGR was due to a combination of large reductions in both NAR and LAR.

In *Capsicum*, the influence of temperature on NAR was accession-dependent. Reduction of NAR at lowered temperatures ranged from 10% for

C. pubescens 'PI 585265' to 28% for *C. chinense* 'PI 543184' (Figure 3.4B). Soldati *et al.* (1999) showed that the temperature response of NAR in highland maize was also accession-dependent. These authors, as well as others, concluded that the temperature at which maximum NAR is reached is related to the temperature in the natural habitat of the plants (Bednarz and Van Iersel, 2001; Hunt and Halligan, 1981; Van Iersel and Lindstrom, 1999). Although *C. pubescens* 'PI 585265', which is known to be cold adapted (Bosland, 1996), showed the smallest decrease in NAR under lowered temperatures, and *C. chinense* 'PI 543184' and *C. annuum* 'Jatilaba', which normally grow under higher temperatures (Table 2.1) showed the highest decrease, we found no general relation between the average daily temperature in the natural or cultivation habitat of the accessions and the reduction in NAR under lowered temperatures. Similarly, the temperature responses of growth and growth related traits between populations of *Hordeum spontaneum* (Van Rijn *et al.*, 2000) or different plant species (Loveys *et al.*, 2002) were poorly related to the temperatures of their natural habitats. Probably, the adaptation of our selected *Capsicum* accessions to their natural habitat is dependent on many environmental conditions, not only air temperature, including light intensity (Bruggink, 1987; Bruggink and Heuvelink, 1987), soil temperature (Dodd *et al.*, 2000) nutrient availability (Villa-Castorena *et al.*, 2003) and the difference between day and night temperature (Nilwik, 1981a; Si and Heins, 1996), which are known to affect growth and growth-related traits of *Capsicum* under controlled conditions.

CONCLUSIONS

Our results illustrate that *Capsicum* accessions respond differently to lowered temperatures. Some accessions were better adapted to lowered temperatures than others, which indicates that there is perspective for breeding for lower temperature tolerance in *Capsicum*. The variation in the reduction of RGR under lowered temperatures was due to changes in both NAR and LAR; DM allocation to the leaves (LMF) was not influenced by temperature. At both temperatures, NAR was the most important factor explaining the variation in

RGR between different the *Capsicum* accessions. From this study, and the work of others, we hypothesise that NAR is the most important factor to explain the variation in RGR within plants of the same genus or species, and between closely-related plant species.

Chapter 4

*Influence of temperature on morphological plant traits and their relationship to relative growth rate in sweet pepper (***Capsicum annuum** *L.) compared to a group of wild and cultivated* **Capsicum** *accessions*

Erik A.M. de Swart, Piet Stam, Roeland E. Voorrips, Leo F.M. Marcelis

Submitted

ABSTRACT

In the Netherlands, sweet pepper (*Capsicum annuum* L.) plants are grown in heated greenhouses. Lowering greenhouse temperature results in substantial reduction in energy use. In order to grow crops under lowered temperatures, cultivars are needed which are adapted to these conditions. In this study we compared the variance in adaptation to low temperatures in cultivated sweet pepper varieties to that of a broad range of wild and cultivated *Capsicum* accessions. The adaptation to lowered temperatures was defined in terms of the relative change in relative growth rate (RGR) between plants grown at different temperature conditions. Our results illustrate that within the group of sweet peppers, the variation for lower temperature tolerance was limited and that sweet pepper breeders should exploit the genetic variation in wild relative species in order to breed for this trait. RGR, however, is difficult and expensive to assess. Therefore, we further studied which morphological plant trait (leaf, stem and total fresh- and dry mass, plant height, leaf area, number of leaves) could be used to simplify the selection of RGR. The relations between RGR and other plant traits could only be demonstrated when a considerable amount of variation for these traits was present in the experimental group of plants. As a result of this, the practical use of these traits as a measure for RGR was limited. Relative leaf growth rate (RLGR) showed to be a good alternative measure for RGR.

INTRODUCTION

In the Netherlands, sweet pepper (*Capsicum annuum* L.) plants are grown in heated greenhouses. To save energy costs, various research has been performed to improve technical measures (reviewed by Bot, 2001) or to advance greenhouse climate control (Körner *et al.*, 2004; Körner and Challa, 2003; Ottosen *et al.*, 2003; Sigrimis *et al.*, 2000). Dieleman *et al.* (2005) showed that in the sweet pepper cultivar 'Solution' six percent of used energy could be saved by climate control measurements compared to the standard climate conditions without significant loss of production or fruit quality. Elings *et al.* (2005) further calculated that by lowering the temperature set point in the greenhouse by two degrees, up to 16% of energy could be saved in glasshouse tomato production. In order to grow crops under lowered temperatures, however, cultivars are needed which are adapted to these conditions.

The ability of plants species to adapt to temperature, which is an important factor determining plant growth and crop production, is species dependent (Beadle *et al.*, 1985). Hekneby *et al.* (2006), for example, found promising variation for low temperature tolerance in a group of annual legume cultivars. In a review Van der Ploeg and Heuvelink (2006) concluded that the variation between chrysanthemum cultivars could be used to breed for more energy-efficient cultivars. In contrast to their observation in chrysanthemum, these authors demonstrated that successful breeding for equal production at lowered temperatures in tomato can best be achieved by exploiting the genetic variation in wild *Lycopersicon* species (Van der Ploeg and Heuvelink, 2005).

In an earlier study, we uncovered some variation for low temperature tolerance within the genus *Capsicum* (De Swart *et al.*, 2006). The adaptation to lowered temperature tolerance was defined in terms of the relative change in relative growth rate (RGR: increase in dry mass (DM) per unit biomass per unit of time; Hunt, 1982) between plants grown at different temperature conditions. Since phenotypic plant traits are highly variable throughout growth and development, comparisons between plant traits are preferably performed at the same developmental stage (Coleman *et al.*, 1994); values for RGR were

therefore compared at the start of the generative growth phase which coincided with the moment of branching.

The results from our earlier study indicated good perspectives for breeding sweet pepper cultivars with low temperature tolerance and increased energy efficiency (De Swart *et al.*, 2006). To produce these kinds of adapted cultivars, plant breeders must be able to identify accessions with increased low temperature tolerance easily in large germplasm collections. Complex physiological traits such as RGR, however, are difficult and expensive to assess (Jackson *et al.*, 1996). Therefore, the first aim of this study was to simplify selection for this complex trait by identifying (morphological) traits that are closely linked to RGR under various temperature regimes and that are indicative for RGR at the moment of branching. On of these traits was the relative leaf growth rate (RLGR; increase in leaf area per leaf area per unit of time; Evans, 1972). The advantage of using RLGR instead of RGR is that it can be calculated based on non-destructive measurements of leaf area (Anchondo *et al.*, 2002). Earlier we showed that in *C. annuum*, leaf area could be accurately estimated indeed from non-destructive measurements (De Swart *et al.*, 2004a). The value of RLGR for breeding purposes will be discussed.

Secondly we compared the variance in low temperature tolerance in cultivated sweet pepper varieties to that of a broad range of wild and cultivated *Capsicum* accessions. This should give an indication about the amount of low temperature tolerance present in the genus *Capsicum* in relation to this variation present in a relatively narrow subgroup of the same genus.

MATERIALS AND METHODS

Experiment 1

Ten *Capsicum* accessions were used, including *C. annuum* 'Bruinsma Wonder' (CGN, Wageningen, The Netherlands); *C. annuum* 'Jatilaba' (RIV, Lembang, Indonesia); *C. chinense* 'PI 159233' (CGN, Wageningen, The Netherlands); *C. annuum* 'PI 585238' (The National Germplasm System, USA); *C. pubescens* 'PI 585265' (The National Germplasm System, USA); *C. baccatum* 'PI 585248' (The National Germplasm System, USA); *C. baccatum*

'PI 585241' (The National Germplasm System, USA); *C. chinense* 'PI 543184' (The National Germplasm System, USA); F_1 '(Jatilaba x Bruinsma Wonder)'; and F_1 '(PI 585238 x Bruinsma Wonder)'. More information about these accessions is given by De Swart *et al.* (2006).

Plants were grown in a glasshouse in Wageningen (The Netherlands, latitude 52 °N) from September 2003 to January 2004. Germination occurred at a day/night temperature of 21°/19°C. At 14 days after sowing (d.a.s.) seedlings were transplanted to rockwool cubes. Germination of seeds, transplanting of seedlings and cultivation was performed as described by De Swart *et al*. (2006). *Capsicum* accessions used in this experiment developed a main stem that ended with one or two flowers and branched into two or three side branches. Branching started between 40 and 60 d.a.s. (standard temperatures) or between 58 and 68 d.a.s. (lowered temperatures). In this study, two of these first order branches were retained. Subsequently, the largest of each higher order branch was retained, while the smallest branch was removed above the first leaf (De Swart *et al.*, 2004a). Flower buds were removed in an early stage of development.

The temperature treatment started at 14 d.a.s. Plants were distributed over four glasshouse compartments. The day started at 6:00 AM and ended at 10:00 PM (day/night 16h/8h). Two compartments had an actual average day/night temperature of 21.1°/18.6 °C and 21.2°/18.7 °C respectively (standard temperature); the other two had an average day/night temperature of 17.3°/14.7 °C and 17.2°/14.7 °C respectively (lowered temperature). The 24-h average temperatures of the different compartments were 20.3° and 20.5° for standard temperatures and 16.5° and 16.4°C for lowered temperatures. Relative air humidity varied from 66% to 69%. Supplementary light (25 µmol m^{-2} s^{-1} PAR) was provided by HPI-T lamps between 6:00 AM and 10:00 PM, when the global radiation outside of the glasshouse was lower than 100 J m^{-2} s^{-1}.

The experiment was arranged in a split-plot design with four whole plots (compartments) and 40 sub plots within each compartment (i.e. four sub plots per accession). The whole experiment was surrounded with border plants of the ten accessions. Each sub plot consisted of two rows of plants, each with one border plant at both ends of each row. The distance between the rows

was 40 cm. A sub plot contained ten experimental plants of which the largest and smallest plant were removed prior to the second harvest to minimize plant-to-plant variation (Poorter, 1989b). At 15, 21, 28, 35, 42, 49 and 63 d.a.s., one randomly chosen plant per sub plot was harvested. After each harvest the remaining plants were rearranged within each sub plot, such that the distance between the plants in a row increased from 11.5 to 40 cm during the experiment. An additional harvest was performed for the lowered temperature treatment at 98 d.a.s. Per harvest, a total number of eight plants per accession were measured for both temperature treatments.

Experiment 2

Six sweet pepper cultivars (*Capsicum annuum* L.) were measured, including four open pollinated cultivars: 'Bruinsma Wonder' (CGN, Wageningen, The Netherlands); 'Lange Rode' (De Bolster, Kielwindeweer, The Netherlands); 'Jumbo' (Saatguthandel Allerleirauh GmbH, Echzell, Germany); 'Rosso' (Saatguthandel Allerleirauh GmbH, Echzell, Germany); and two hybrid varieties: 'Bendigo F_1' and 'Sprinter F_1' (Vitalis Biologische Zaden BV, Voorst, The Netherlands).

Plants were grown in climate rooms. During the whole experiment, light (200 (± 7.5%) µmol m^{-2} s^{-1} PAR) was provided by HPI-T lamps between 6:00 AM and 10:00 PM. Light intensities were checked and adjusted weekly. Relative air humidity was set at 70%. Seeds were sown in trays (25 x 25 x 40 mm $plug^{-1}$) containing an organic soil mixture (Lentse Potgrond B.V., the Netherlands). Trays were daily moistened with water. Germination occurred at a day/night temperature of 21°/18°C. At 14 days after sowing (d.a.s.) seedlings were transplanted to pots with a diameter of 17 cm, containing the same organic soil mixture. From that moment on, pots were watered daily and CO_2 was applied (500 ppm CO_2).

The temperature treatment started at 14 d.a.s. Plants were distributed over four climate rooms (day/night 16h/8h). In two climate rooms, temperature was set at 21°/18°C (standard temperature); the other two had a set temperature of 18°/14°C (lowered temperature). Plants were pruned as described above. The nutrient availability of the soil mixture was checked once

during the experiment and once at the end of the experiment. No depletion of nutrients had occurred during the experiment.

The experiment was arranged in a randomised block design with two blocks (compartments) at standard temperatures and two blocks at lowered temperatures. Each block contained 20 plants per accession. Prior to the first harvest (25 d.a.s.), the largest and smallest plants were removed to minimize plant-to-plant variation, leaving 16 plants per accession within each block. At 29, 40, 50 and 61 d.a.s. (standard temperatures) or 29, 40, 61 and 77 d.a.s. (lowered temperatures), four randomly chosen plants per accession and per block were harvested. After each harvest the remaining plants within each block were rearranged. The plant density decreased from 20 plants per m^2 at the start of the experiment (15 d.a.s.) to 1 plant per m^2 prior to the final harvest. Per harvest, a total number of eight plants per accession were measured for both temperature treatments.

Measurements

At each harvest, plant height was measured as well as the height of the branching point and the number of leaves above and below the branching point. Subsequently, plants were separated into two fractions: leaf blades and remaining above ground fraction, consisting of the stems and petioles. Fresh masses of each fraction as well as the areas of the leaf blades (Licor LI-3100 leaf area meter) were determined. Dry masses were determined on oven-dried (48 h at 70 °C) material. Time of branching was recorded as well. Plant height was measured with a ruler. The morphological traits that will be presented are: stem, leaf and total fresh and dry mass; leaf area; plant height, number of leaves and internode length.

Calculations and statistical analysis

Relative growth rate (RGR) was calculated for each time interval (eight plants per time point) as described by Hunt *et al.* (2002); RLGR was calculated as described by Evans (1972). Values of RGR and RLGR at the moment of branching were calculated as described by De Swart *et al.* (2006). All statistical analyses were performed using the Genstat 6.0. statistical package (Payne *et al.*, 2002). Relationships between RGR and morphological growth

characteristics, RGR and RLGR, and morphological growth traits under different temperatures were analysed by linear regression analysis. Analysis of Variance (ANOVA) was used to determine the effect of temperature on morphological growth traits.

RESULTS

Effect of temperature on plant morphological traits

The relation between RGR at the moment of branching and a number of morphological plant traits was analysed in 'Experiment 1' in which a group of ten different wild and cultivated *Capsicum* accessions were grown at two contrasting temperature regimes (21.1°/18.7°C and 17.3°/14.7°C). One day after the start of the temperature treatment (15 d.a.s.), none of the morphological traits of plants of the same accession were significantly different between the temperature treatments. Within both temperature treatments, significant differences in fresh and dry masses between different accessions were already visible. ANOVA showed that from 21 d.a.s. on, temperature had a significant effect on all measured morphological traits for all accessions ($P < 0.001$). Plants grown under standard temperatures had a significantly higher leaf, stem and total fresh mass (Figure 4.1A-B), leaf, stem and total dry mass (Figure 4.1C-D) and leaf area (Figure 4.1E-F) compared to plants of the same accessions grown under lowered temperatures. These plants also developed more leaves (Figure 4.1G-H), longer stems (both main stem and total plant height; Figure 4.1I-J) and had longer internodes (Figure 4.1K-L) than plants at lowered temperature.

Regression analysis showed that from 28 d.a.s onwards values of all measured plant morphological traits of plants of the different accessions grown under standard temperatures were positively correlated to those at lowered temperatures (Table 4.1).

Table 4.1: *Correlations (R^2) between various morphological plant traits in ten different Capsicum accessions (Experiment 1) at standard (21.1/18.7°C) and lowered (17.3/14.7°C) temperatures at different moments in time (days after sowing). Significance levels are indicated as 0.10<P<0.05 (*), 0.05 < P<0.01(**) or P<0.01 (***)*

Trait	28 d.a.s.		35 d.a.s.		42 d.a.s.		49 d.a.s.		63 d.a.s.	
Fresh mass leaf	0.91	***	0.94	***	0.89	***	0.93	***	0.82	***
Fresh mass stem	0.92	***	0.96	***	0.84	***	0.89	***	0.81	***
Total fresh mass	0.93	***	0.97	***	0.90	***	0.94	***	0.83	***
Dry mass leaf	0.34	*	0.93	***	0.87	***	0.92	***	0.84	***
Dry mass stem	0.92	***	0.93	***	0.88	***	0.92	***	0.82	***
Total dry mass	0.78	***	0.93	***	0.86	***	0.93	***	0.82	***
Leaf area	0.92	***	0.86	***	0.84	***	0.89	***	0.81	***
Number of leaves	0.65	***	0.64	***	0.78	***	0.86	***	0.89	***
Plant height	0.81	***	0.71	***	0.73	***	0.80	***	0.76	***
Internode length	0.58	***	0.61	***	0.77	***	0.59	***	0.65	***

Relation between RGR and plant morphology

To determine if and at which point in time the different plant morphological traits are indicative for RGR at the moment of branching, the relation between the various morphological traits and RGR at the moment of branching was determined in the group of wild and cultivated *Capsicum* accessions for each morphological trait at each measuring point; RGR values are shown in Figure 4.2.

Significant correlations between RGR at the moment of branching and morphological growth characteristics were found at both temperature treatments after each individual plant had reached a total dry mass of at least 50 mg. Under standard temperatures this dry mass was reached for all accessions at 35 d.a.s. From this moment on, plant fresh mass (leaf, stem and total) remained correlated to RGR (Table 4.2). Similar results were found for plant dry mass (stem, leaf, total) and leaf area. The correlation between RGR

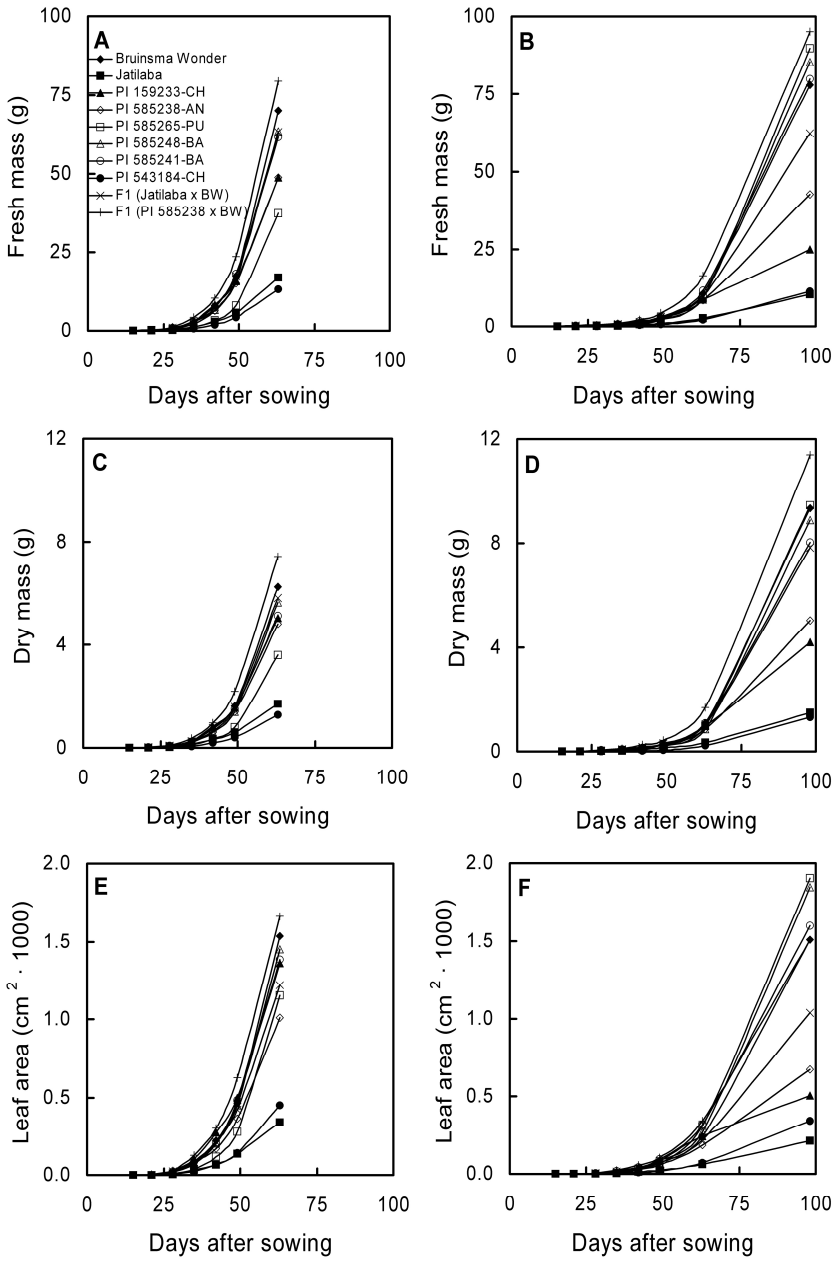


Figure 4.1: *(A, B) Total fresh mass; (C, D) total dry mass; (E, F) leaf area; (G, H) number of leaves; (I, J) plant height and (K, L) internode length over time (days after sowing) in ten Capsicum accessions (Experiment 1) grown under (A, C, E, G, I, K) standard (21.1°/18.7°C); or (B, D, F, H, J, L) lowered (17.3°/14.7°C) temperatures.*

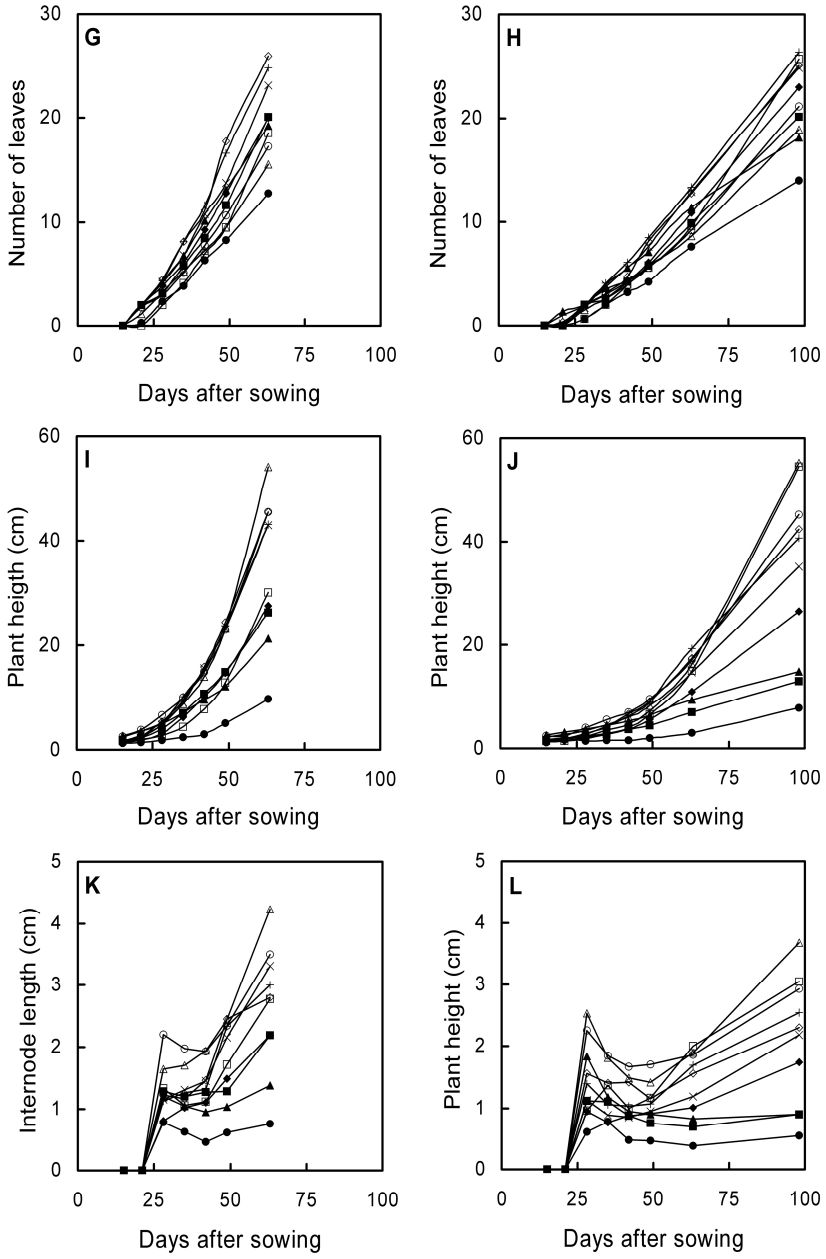


Figure 4.1 continued

and the total number of leaves became significant ($P < 0.05$) after 42 days. At no moment in time, correlations between RGR and plant height, the length of the main stem and the internode length were significant. F_1 '(PI 585238 x Bruinsma Wonder)', the accession with the highest RGR at the moment of branching, also had the highest fresh- and dry mass (stem, leaf, total; Figure 4.1A, C) and the largest leaf area (Figure 4.1E) of all accessions during the whole experiment.

Under lowered temperatures the total dry mass of 50 mg was reached at 49 d.a.s. for all accessions. From 49 d.a.s. on, plant fresh mass (leaf, stem and total) was continuously correlated to RGR. Similar results were found for leaf area, leaf dry mass and total dry mass; Stem dry mass and the number of leaves were only related to RGR at the moment of branching at final harvest (98 d.a.s.). Although F_1 '(PI 585238 x Bruinsma Wonder)' was not the accession with the highest RGR under lowered temperatures, this accession had the highest fresh and dry mass (stem, leaf, total) and the highest leaf area of all accessions at all measuring points (Figure 4.1B, D, F). The accession with the highest RGR under lowered temperatures, *C. pubescens* 'PI 585265', had a lower fresh- and dry mass (stem, leaf, total) and leaf area during the first 49 days of the experiment. After 49 days (ten days before branching), this accession showed a relatively higher increase in fresh- and dry mass (stem, leaf, total) and leaf area compared to other accessions. At final harvest *C. pubescens* 'PI 585265' had the highest values for fresh- and dry mass (stem, leaf, total) and leaf area after F_1 '(PI 585238 x Bruinsma Wonder)'.

Relation between relative growth rate and relative leaf growth rate

A second parameter for growth is the relative leaf growth rate (RLGR) that describes the increase of leaf area in time. In cases where destructive measurement cannot be used this parameter might be used as an alternative for RGR if the two quantities are sufficiently correlated. Values of RLGR at the moment of branching varied between 0.0876 $cm^2\ cm^{-2}\ d^{-1}$ for ' Jatilaba' to 0.1451 $cm^2\ cm^{-2}\ d^{-1}$ for F_1 '(PI 585238 x Bruinsma Wonder)' under standard temperatures (Figure 4.2A); under lowered temperatures RLGR ranged from 0.0445 $cm^2\ cm^{-2}\ d^{-1}$ for *C. chinense* 'PI 159233' to 0.1158 $cm^2\ cm^{-2}\ d^{-1}$ for *C. pubescens* 'PI 585265' (Figure 4.2B). The difference in RLGR between the

Table 4.2: *Correlations (R^2) between RGR at the moment of branching and various plant morphological traits of Capsicum accessions grown under standard (21.1/18.7°C) or lowered (17.3/14.7°C) temperatures at different days after sowing. Significance levels are indicated as not significant (n.s.), 0.10 < P < 0.05 (*), 0.05 < P< 0.01(**) or P < 0.01 (***). Data derived from the 'Experiment 1'*

Trait	35		42		49		63		98	
Standard temperatures										
Fresh mass leaf	0.47	**	0.37	**	0.47	**	0.53	**		
Fresh mass stem	0.51	**	0.47	**	0.45	**	0.46	**		
Total fresh mass	0.51	**	0.45	**	0.5	**	0.55	***		
Dry mass leaf	0.5	**	0.4	**	0.51	**	0.58	***		
Dry mass stem	0.41	**	0.44	**	0.51	**	0.54	***		
Total dry mass	0.5	**	0.46	**	0.56	**	0.63	***		
Leaf area	0.51	**	0.43	**	0.51	**	0.56	***		
Number of leaves	0.28	*	0.35	**	0.37	**	0.42	**		
Lowered temperatures										
Fresh mass leaf		n.s.		n.s.	0.25	*	0.38	**	0.55	***
Fresh mass stem		n.s.		n.s.	0.21	*	0.23	**	0.56	***
Total fresh mass		n.s.		n.s.	0.25	**	0.33	**	0.57	***
Dry mass leaf		n.s.		n.s.	0.28	**	0.38	**	0.56	***
Dry mass stem		n.s.		n.s.		n.s.		n.s.	0.52	***
Total dry mass		n.s.		n.s.	0.24	*	0.33	**	0.57	***
Leaf area		n.s.		n.s.	0.28	**	0.47	**	0.51	**
Number of leaves		n.s.		n.s.		n.s.		n.s.	0.53	**

fastest en the slowest growing accession was 40% under standard temperatures and 62% under lowered temperatures. High and significant correlations (P < 0.001) were found between RGR and RLGR at both standard (R^2 = 0.93) and lowered temperatures (R^2 = 0.91). The relation of RLGR and

RGR could be described as RLGR = 0.93 • RGR) under standard temperatures. Under lowered temperatures it reads RLGR = 1.21 • RGR - 0.021. ANOVA further showed that RGR and RLGR were not significantly different ($P > 0.1$) at neither of the temperature regimes. RLGR of accessions grown under standard temperatures was positively correlated ($R^2 = 0.53$; $P = 0.01$) to RLGR under lowered temperatures. For RGR the correlation was similar ($R^2 = 0.58$; $P = 0.007$).

Variation in RGR and growth related traits among sweet pepper cultivars

The influence of temperature regime (21°/18°C or 18°/14°C) on growth and development was further investigated in a narrow subgroup of the genus *Capsicum*, namely a group of sweet peppers (*C. annuum*). By using this subgroup a comparison could be made between the variation for low temperature tolerance in cultivated sweet pepper varieties and the same variation in a broad group of *Capsicum* accessions.

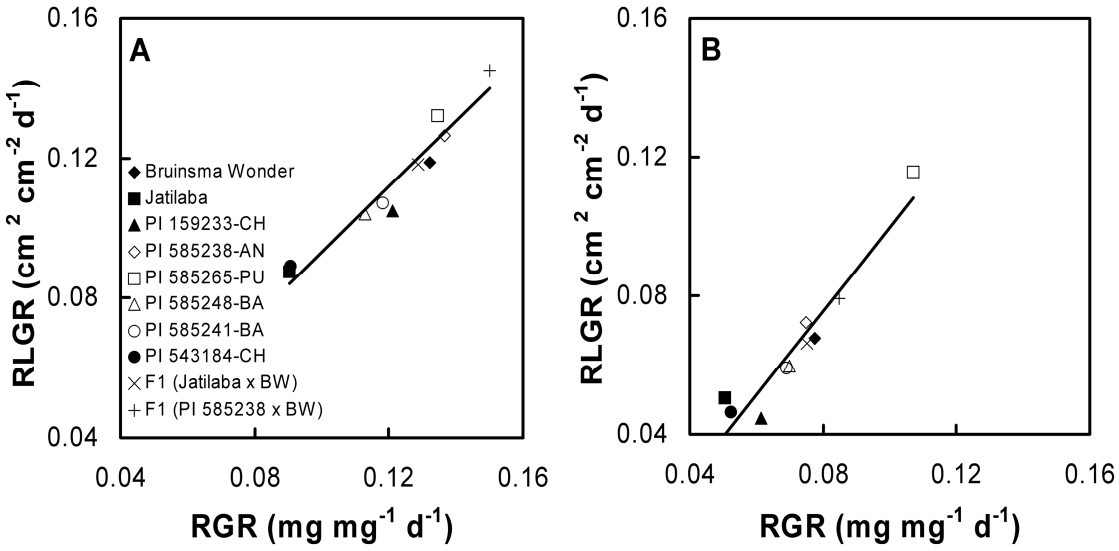


Figure 4.2: *Relation between mean values of relative growth rate (RGR) at the moment of branching and relative leaf growth rate (RLGR) at the moment of branching for ten Capsicum accessions (Experiment 1) grown under (A) standard (21.1°/18.7°C); or (B) lowered (17.3°/14.7°C) temperatures ($P < 0.01$). RGR values are taken from (De Swart* et al.*, 2006).*

Sweet pepper plants grown under standard temperatures had a significantly higher leaf, stem and total dry mass, leaf, stem and total fresh mass and leaf area compared to plants of the same accessions grown under lowered temperatures at all measurements ($P < 0.001$). Plant height, the number of leaves and the internode length were also significantly smaller at lowered temperatures.

Values of RGR at the moment of branching varied between 0.1430 g g^{-1} d^{-1} for 'Bruinsma Wonder' to 0.1611 g g^{-1} d^{-1} for 'Bendigo F_1' under standard temperatures (Figure 4.3A); under lowered temperatures RGR ranged from 0.0686 g g^{-1} d^{-1} for 'Bruinsma Wonder' to 0.0874 g g^{-1} d^{-1} for 'Bendigo F_1' (Figure 4.3B). The difference in RGR between the fastest en the slowest growing accession within the group of sweet pepper cultivars was 13% under standard temperatures and 22% under lowered temperatures. The average reduction in RGR between the temperature treatments was 46%. The reduction varied between 43% for 'Jumbo' and 'Sprinter F_1' and 52% for 'Bruinsma Wonder'.

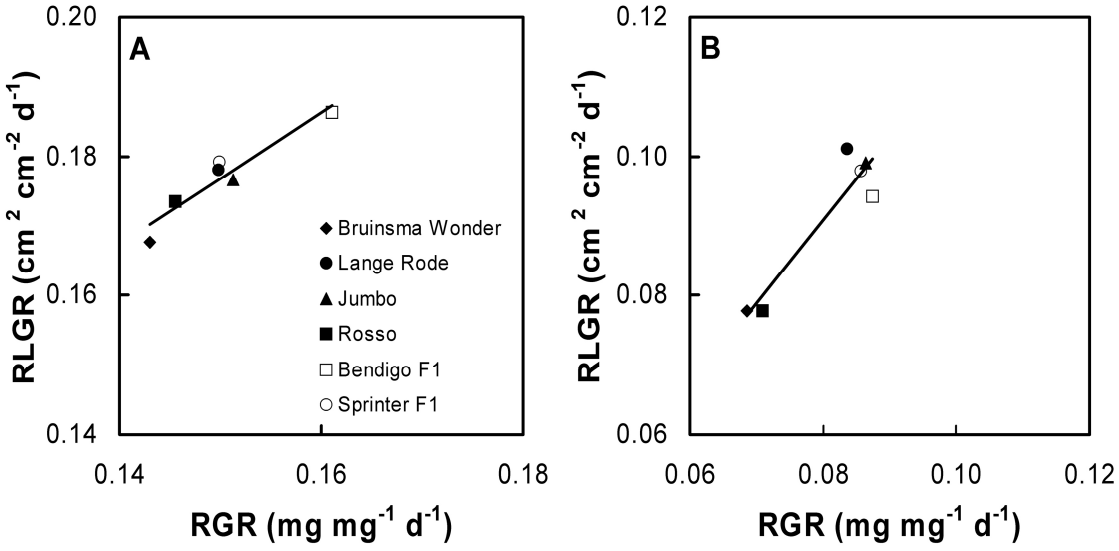


Figure 4.3: *Relation between mean values of relative growth rate (RGR) at the moment of branching and relative leaf growth rate (RLGR) at the moment of branching for six sweet pepper cultivars (Experiment 2) grown under (A) standard (21°/18°C); or (B) lowered (18°/14°C) temperatures (P < 0.01).*

RLGR varied 10% under standard temperatures and 23% under lowered temperatures. Values of RLGR at the moment of branching varied between 0.1676 $cm^2\ cm^{-2}\ d^{-1}$ for 'Bruinsma Wonder' to 0.1863 $cm^2\ cm^{-2}\ d^{-1}$ for 'Bendigo F_1' under standard temperatures; Under lowered temperatures RLGR ranged from 0.0777 $cm^2\ cm^{-2}\ d^{-1}$ for 'Bruinsma Wonder' to 0.1011 $cm^2\ cm^{-2}\ d^{-1}$ for 'Lange Rode'.

The ratios between RLGR and RGR were different for both temperature treatments; under standard temperatures RLGR = 0.95 • RGR + 0.035 (R^2 = 0.90; $P < 0.001$) and at lowered temperatures RLGR = 1.14 • RGR (R^2 = 0.87; $P < 0.001$). ANOVA further showed that values for RGR and RLGR were not significantly different ($P > 0.1$) at lowered temperatures, whereas RLGR was significant higher than RGR under standard temperatures ($P < 0.001$).

DISCUSSION

Both in the group of wild and cultivated *Capsicum* accessions, containing only one sweet pepper variety (Experiment 1; Figure 4.1), and in the group of sweet pepper cultivars (Experiment 2), values for plant morphological characteristics are influenced by temperature. Other studies in *Capsicum* also show that values for dry mass, fresh mass, leaf area, plant height, number of leaves and internode length decrease when plants are exposed to lowered temperatures (Bakker and Van Uffelen, 1988; Deli and Tiessen, 1969; Mercado *et al.*, 1997; Nilwik, 1980a; Pressman *et al.*, 2006; Si and Heins, 1996).

Similar results were found in other species: In alfalfa, goatsrue (Patterson, 1993), *Bellis perennis*, *Dactylis glomerata*, *Poa annuum* (Gunn and Farrar, 1999) and southern Beech (Hovenden, 2001) for example, dry mass and leaf area decreased as a result of a lowered temperature. Hurd and Graves (1985) found in tomato that plant height was reduced at lower temperatures, as well as the number of leaves.

The high correlations between morphological traits of plants grown under different temperatures (Table 4.1) show that measurement of plant traits under standard temperatures give a good indication for the characteristic under lowered temperatures. For practical use this means that for a first

screening of morphological traits that influence growth under lowered temperatures at a certain moment in time, measurements can be performed under standard temperatures.

Limited variation within sweet pepper

As expected, the variation for growth and growth related traits was much higher in the group of wild and cultivated *Capsicum* accessions than within the group of sweet pepper cultivars. In the group of wild and cultivated *Capsicum* accessions the difference in total dry mass between the largest and smallest accession at final harvest was 83% (Figure 4.1C) under standard temperatures (63 d.a.s.) and 88% under lowered temperatures (98 d.a.s.; Figure 4.1D); within the group of sweet pepper varieties this difference was limited to 33% and 43% under standard (61 d.a.s.) and lowered temperature treatment (77 d.a.s.) respectively. With respect to RGR, similar results were found. These differences were 39% (standard temperature treatment) or 53% (lowered temperatures) in the experiment containing a wide range of wild and cultivated *Capsicum* accessions. The group of sweet pepper cultivars showed a difference in RGR of 13% under standard temperatures and of 22% under lowered temperatures between the fastest and slowest sweet pepper cultivar.

In an earlier study we found that within a wide range of *Capsicum* accessions, some accessions were better adapted to lowered temperatures than others, indicating a perspective for breeding for lower temperature tolerance in *Capsicum* (De Swart *et al.*, 2006). Experiment 2 showed that within the group of sweet pepper cultivars, the variation for lower temperature tolerance was limited: the difference in RGR between the two temperature treatments varied between 43% and 52%. In the study described by De Swart *et al.* (2006) the range of this difference was much broader (20% - 49%). These findings correspond to those of Van der Ploeg and Heuvelink (2005) who concluded in their review that the variation for lowered temperature response in cultivated tomato was limited also. Our results therefore indicate that, like in tomato, also in sweet pepper breeders should exploit the genetic variation in wild relative species in order to breed cultivars with increased energy efficiency.

Relation between RGR and plant morphology

As a consequence of the limited variation for growth and growth related traits within the group of sweet pepper cultivars, the relations between RGR and morphological plant traits that were found in the group of wild and cultivated *Capsicum* accessions (Table 4.1) could not be identified in the sweet pepper group. The fact that the relation between RGR at the moment of branching and the various morphological plant traits is dependent on the amount of variation present for these traits in the experimental group of plants, reduces the practical use of these traits as a measure for RGR dramatically.

In breeding programmes this variation is often limited. Paran *et al.* (1998), for example, showed that there is only limited variation within the group of blocky type cultivars. Selection based on RGR or morphological plant traits correlated to RGR is therefore not useful in these cases. To be able to select for variation in RGR and growth related morphological traits, breeders should focus on variation present in wild material.

Relation between relative growth rate and relative leaf growth rate

In *Capsicum*, RLGR at the moment of branching was shown to be an excellent predictor for RGR at the same developmental stage. Evans (1972) described RLGR as a measure for leaf area growth in time analogous to RGR. At the moment of branching RGR and RLGR were strongly correlated under the standard and lowered temperature treatment in both experiments (Figure 4.2 and 4.3), even when the variation in RGR was only limited (Figure 4.3B). The ratios between RGR and RLGR were close to one and, except for the ratio between RGR and RLGR in the sweet pepper experiment at standard temperatures, not significantly different from unity. High correlations between RGR and RLGR were earlier found among species (Potter and Jones, 1977) and in *Brassica* (Paul, 1992). The great advantage of using RLGR is that it can be calculated based on data of non-destructive measurements of leaf area. Especially in breeding programs and genetic research, where unique plants in segregating populations are used, non-destructive measurements can be of great value. In these cases RLGR is a good alternative for RGR.

CONCLUSIONS

Our results illustrate that within the group of sweet pepper cultivars, the variation for lower temperature tolerance is limited. Sweet pepper breeders should exploit the genetic variation in wild *Capsicum* species in order to breed cultivars with increased energy efficiency. The relation between RGR at the moment of branching and the various morphological plant traits is dependent on the amount of variation present for these traits in the experimental group of plants. As a result of this the practical use of these traits as a measure for RGR is limited. A good alternative measure for RGR is RLGR.

Chapter 5

QTLs for growth and growth related traits in Capsicum annuum *L.*

Erik A.M. de Swart, Remmelt Groenwold, Piet Stam, Roeland E. Voorrips

Plant Breeding: accepted with revision

ABSTRACT

The aim of this study was to identify QTLs with an effect on plant morphology, growth and growth related traits in *Capsicum annuum*. In an intraspecific F_2 population, a genetic map with a length of 817 cM was constructed based on AFLP markers. The map consisted of 16 linkage groups, two of which could be assigned to chromosomes. A total number of 18 QTLs with an effect on plant morphology and growth were identified by multiple-QTL mapping analysis (LOD > 3.0). One QTL for fruit shape on chromosome 3 (linkage group 1) co-incided with a QTL described earlier. The effect of seven other QTLs related to plant growth and development (e.g. relative leaf growth rate, leaf mass fraction, dry mass, number of leaves) could be established in a group of 25 selected F_3 lines. We concluded that a relatively small F_2 population can be of great value for studying growth and growth related QTLs provided that it is combined with confirming the detected QTLs in, for example, an F_3 generation.

INTRODUCTION

Capsicum is a large genus that belongs to the Solanaceae family (Heywood, 1978). It has an enormous richness in diversity (Bosland, 1992) and appears in many different cultivars, forms and uses (Bosland and Votava, 2000). Whereas in the past genetic diversity and inheritance could only be studied based on phenotypic analysis (Heiser and Smith, 1953), a new era opened up by the development of molecular biology over the last decades, making it easy to assess genetics behind the diversity within the *Capsicum* species. Using molecular techniques, Paran *et al.* (1998) showed that there is only a limited variation within the group of blocky type cultivars, whereas the group of small-fruited cultivars is more diverse.

Next to genetic diversity studies, molecular tools are also very useful in Quantitative Trait Locus (QTL) analysis. The identification of QTL regions allows breeders to use marker-assisted selection to precisely transfer beneficial QTL alleles into agricultural strains for crop improvement. In various recent studies it has been found that QTLs accurately position the genes underlying polygenic traits on the genome (Price, 2006), especially in case of major QTLs (Salvi and Tuberosa, 2005).

In *Capsicum*, QTLs have been identified for important traits like disease resistance (Ben Chaim *et al.*, 2001a; Caranta *et al.*, 1997, 2002; Lefebvre *et al.*, 2003; Quirin *et al.*, 2005; Thabuis *et al.*, 2004; Voorrips *et al.*, 2004), pest resistance (Djian-Caporalino *et al.*, 2001) fruit quality and shape (Ben Chaim *et al.*, 2001b, 2003a, b; Huh *et al.*, 2001; Lefebvre *et al.*, 1998; Zygier *et al.*, 2005), fertility restoration (Wang *et al.*, 2004) and yield (Rao *et al.*, 2003). For plant growth no QTLs were identified in *Capsicum* yet and the number of QTLs for growth related characteristics is limited (Ben Chaim and Paran, 2000). This may be due to the fact that plant growth is an adaptive trait that is easily influenced by the environment (Grime and Hunt, 1975). An other reason could be that plant growth may be controlled by many genes with small individual effects that are difficult to detect (Courtois *et al.*, 2000; Remington and Purugganan, 2003; Van Rijn, 2001).

The aim of this study was to identify QTLs with an effect on plant morphology, growth and development that could be used in breeding programmes for better growth under lowered temperatures. In a review Maloof (2003) concluded that there is a substantial interest in using QTL mapping to understand the genetic basis of variation in plant growth and morphology. Slafer (2003) argued that molecular tools like QTL mapping can be beneficial to breeding for yield when adequate physiological understanding of the determination of yield by relatively simple traits (e.g. plant height) is present. In this study we focussed on the genetics of traits involved in growth and development of the plants. To find QTLs for growth and growth related traits we observed an F_2 population of an intraspecific *C. annuum* cross. We measured both growth and easily measurable determinants of growth and development, performed a marker analysis, constructed a linkage map and performed QTL analysis. The effects of the QTLs identified in the F_2 population were validated in a set of F_3 lines where more individual (F_3) plants represented the genotype of a single F_2 plant.

MATERIALS AND METHODS

Plant material

Capsicum annuum L. 'Jatilaba' (RIV Lembang, Indonesia) is a slow growing Indonesian open-pollinated (OP) hot pepper variety; *C. annuum* 'Bruinsma Wonder' (CGN, Wageningen, The Netherlands) is Dutch fast growing OP sweet pepper variety with block-type fruits (De Swart *et al*., 2004b, 2006). *C. annuum* 'Nassau F_1' (Rijk Zwaan, De Lier, The Netherlands) is a Dutch hybrid sweet pepper with block-type fruits and is known for its vigorous growth. A Jatilaba x Bruinsma Wonder F_2 population was obtained from one single self-pollinated F_1 plant. F_2 plants were self-pollinated yielding 127 F_3 lines. A subset of 25 F_3 lines was selected based on the homozygous presence of the Bruinsma Wonder or the Jatilaba allele of the QTL for relative leaf growth rate (RLGR; Evans, 1972).

F_2 experiment

150 F_2 (Jatilaba x Bruinsma Wonder), 20 'Jatilaba', 20 'Bruinsma Wonder' and 20 'Nassau F_1' plants were grown as described in Chapter 2 from December 2000 to March 2001. After transplanting, plants were divided into four blocks containing 37 or 38 F_2 plants and five plants each of the parental lines and 'Nassau F_1'. Parental and 'Nassau F_1' plants were randomly distributed within each block. Blocks were surrounded with border plants of the same accessions used in the experiment. Temperature was set at 21 °C during daytime (16 hours) and 18 °C at night (eight hours). The average daily global radiation outside of the glasshouse was 3.4 MJ m^{-2} d^{-1}, ranging from 0.4 to 12.5 MJ m^{-2} d^{-1}. Artificial HPI-T light (25 µmol m^{-2} s^{-1} PAR) was added during daytime. Relative air humidity varied from 50 to 70%. Leaf length and width were measured non-destructively at 39, 49, 60 and 69 days after sowing (d.a.s.). Prior to the final harvest, cuttings were made to maintain the F_2 plants. At final harvest (75 d.a.s.) plant height, height of the branching point and leaf and stem dry mass were determined. From three leaves per plant leaf length, width and area were measured separately to validate a model for estimating leaf area from leaf length and width measurements (De Swart *et al.*, 2004b) in the current population. Dry mass of these leaves was measured to calculate Specific Leaf Area (SLA).

F_3 experiment

200 F_3 (Jatilaba x Bruinsma Wonder) plants (eight plants each of 25 F_3 lines), 16 'Jatilaba' and 16 'Bruinsma Wonder' plants were grown as described above from December 2001 to March 2002. After transplanting the experiment was arranged in a randomised block design with four blocks; the experiment was surrounded with border plants of the same accessions used in the experiment. Each block contained 58 plants: two plants of each F_3 line and four plants of both parental lines. The actual average day temperature in the glasshouse was 22.5 °C (16 hours) and the night temperature was 18.0 °C (8 hours). The average daily global radiation outside of the glasshouse was 3.3 MJ m^{-2} d^{-1}, ranging from 0.4 to 8.9 MJ m^{-2} d^{-1}. Artificial HPI-T light (25 µmol m^{-2} s^{-1} PAR) was added during daytime. Leaf length and leaf width were determined non-destructively at 40, 49, 57 and 63 d.a.s. At final harvest (68 d.a.s.), leaf area

was measured with an area meter (Licor LI-3100; Licor, Lincoln, NE, USA) and plant height, height of the branching point and leaf and stem fresh and dry mass were determined. From five leaves per plant leaf length, width and area were measured separately to validate a model for estimating leaf area from leaf length and width measurements (De Swart *et al.*, 2004b).

Measurements and calculations

Leaf area estimates were obtained from leaf length (L) and leaf width (W) using the model AREA = 0.6190•L•W + 0.2060•W^2 – 0.5142•W (De Swart *et al.*, 2004a). Validation of the model in both the F_2 and F_3 experiments showed high correlations (R^2=0.99) between measured and calculated leaf area and ratios between measured and calculated area of 1.00 and 0.96 respectively.

Total plant height and height of the branching point were measured with a ruler at final harvest; fruit length and fruit width (two fruits per F_2 plant) were also measured with a ruler on F_2 plants propagated from cuttings. Internode length was calculated by dividing the height of the branching point by the number of leaves on the main stem. After harvest, plant material was oven-dried for 48 hours at 70 °C and dry masses of stem and leaves were determined.

SLA and leaf mass fraction (LMF) were calculated as described by Hunt (1982); relative leaf growth rate (RLGR) was calculated based on single plants as described by Evans (1972). Wide sense heritability in the F_2 population was calculated as described by Briggs and Knowles (1967). For heritability calculations, the non-genetic variance was estimated as the average variance of 'Jatilaba', 'Bruinsma Wonder' and 'Nassau F_1'. Year x genotype interactions were tested in a two way ANOVA with data of 'Jatilaba' and 'Bruinsma Wonder'. The different years represent the F_2 and F_3 experiment.

Marker analysis, map construction and QTL mapping

Leaf material of plants grown from cuttings from the original F_2 plants was used for DNA analysis. AFLP markers were generated by Keygene N.V. (Wageningen, the Netherlands) using the procedures of Vos *et al.* (1995). The selective nucleotides for the AFLP primers used in this study were combinations of *Eco*RI and *Mse*I and *Pst*I and *Mse*I primers. Pre-amplification

primers (5' to 3'): E00=GAC TGC GTA CCA ATT C; P00=GAC TGC GTA CAT GCA G; M02= GAT GAG TCC TGA GTA AC. Amplification primers: E32=E00-AAC; E38=E00-ACT; P11=P00-AA, P14=P00-AT; P17=P00-AC; P21=P00-GG; M47=M02-AA; M48=M02-AC; M49=M02-AG; M50=M02-AT; M51=M02-CA; M60=M02-TC; M61=M02-TG. The primer combinations used were: E32M48, E38M49, E38M51, P11M47, P11M48, P11M49, P11M60, P14M47, P14M48, P14M50, P14M61, P17M48, P17M50, P21M50 and P21M61. AFLP fragments were scored as co-dominant markers. Markers are presented as the primer combination followed by the size of the fragments. A linkage map was calculated from the marker data using the software package Joinmap 3.0 (Van Ooijen and Voorrips, 2001).

To identify potential QTL regions, Kruskal-Wallis and interval mapping analyses were performed using the MapQTL program (Van Ooijen *et al.*, 2002). Putative QTLs were identified by using different combinations of markers in the potential QTL regions as co-factors in multiple-QTL mapping analysis (MQM; Jansen and Stam, 1994). The genome wide significance for each QTL was empirically determined by performing 1000 iterations using the permutation test of MapQTL.

In a set of F_3 lines we attempted to validate the QTLs identified in the F_2 population. The F_2 plants were grouped according to QTL genotype: homozygous for either parental allele or heterozygous. A QTL was considered as validated if significant differences occurred among the phenotypic means of the genotypic F_2 groups and the corresponding groups of F_3 lines differed in the same way. All statistical analyses were performed using the Genstat 6.0. statistical package (Payne *et al.*, 2002). The QTL graphs were prepared with MapChart 2.1 (Voorrips, 2002).

Table 5.1: *Average values, variances and heritability (percentage) of the traits measured in 'Jatilaba' (n=20), 'Bruinsma Wonder' (n=20) 'Nassau F_1' (n=20) and F_2 '(Jatilaba x Bruinsma Wonder)' (n=142). Leaf area (LA) was estimated and number of leaves (NRLV) were measured at 39, 49, 60 and 69 d.a.s. At final harvest stem, leaf and total dry mass, plant height, height of the branching point and the number of leaves on the main stem were measured; Specific leaf area (SLA) and leaf mass fraction (LMF) were calculated. Relative leaf growth rate (RLGR) was calculated over the time interval from 39 to 69 d.a.s. Leaf length: width ratio (L:W-ratio) was calculated from data of all length and width measurement*

	Jatilaba		Bruinsma Wonder		Nassau F_1		F_2 (JA x BW)		Heritability
Trait	**Average**	**Variance**	**Average**	**Variance**	**Average**	**Variance**	**Average**	**Variance**	**(%)**
LA_39 (cm^2)	16.9	14	36.9	31	38.4	111	33.5	82	**37**
LA_49 (cm^2)	57.6	164	153.9	561	151.6	1251	124.3	1237	**47**
LA_60 (cm^2)	174	1528	517	6305	486	10196	372	10597	**43**
LA_69 (cm^2)	318	5240	1074	19533	976	29052	737	35467	**49**
Dry mass leaf (g)	1.39	90	3.89	258	3.32	310	2.77	536	**59**
Dry mass stem (g)	1.6	154	4.25	347	4.37	535	3.6	1037	**67**
Total dry mass (g)	2.99	471	8.13	1173	7.7	1561	6.37	2784	**62**
RLGR ($cm^2\ cm^{-2}\ d^{-1}$)	0.0977	0.000025	0.1107	0.000041	0.1072	0.000038	0.1017	0.00004	**14**
SLA ($m^2\ kg^{-1}$)	34.5	2.04	38.4	2.26	39.7	3.18	37.9	10.71	**77**
LMF ($g\ g^{-1}$)	0.47	0.0002	0.48	0.0002	0.43	0.0004	0.44	0.0019	**87**
NRLV_39	4.2	0.10	4.1	0.06	4.1	0.09	4.8	0.85	**89**
NRLV_49	7.7	0.60	7.5	0.51	7.9	0.66	8.7	2.08	**72**
NRLV_60	12.8	1.15	13	0.93	12.8	3.33	14.9	9.44	**81**
NRLV_69	19.1	5.99	19.3	2.72	22.3	9.09	22.7	20.44	**71**
Height (cm)	44.3	15.33	46.3	6.38	48.6	10.44	55.1	75.37	**86**
Branching height (cm)	41.7	8.71	42.9	5.01	40.3	5.49	48	128	**95**
Time till branching (d.a.s.)	60.46	6.58	58.61	2.49	54.3	5.79	56.49	52.81	**91**
NRLV on the main stem	12.39	0.84	12.06	1.56	9.68	0.67	12.33	6.79	**85**
Internode length (cm)	3.35	0.14	3.58	0.11	4.17	0.12	4.01	0.95	**87**
L:W-ratio leaf	2.47	0.01	1.56	0.00	1.52	0.01	2.03	0.04	**89**
Fruit length (cm)							10.5	3.99	
Fruit L:W-ratio							3.1	0.73	
Fruit width (cm)							3.5	0.32	

Table 5.2: *List of QTLs detected in the F_2 population of the cross between* C.annuum *'Jatilaba' and 'Bruinsma Wonder'*

Code	Trait	Marker	Parent[a]	Linkage group	LOD	Significance[b] P	Averages[c] JA	H	BW	%exp[d]
LA	LA_60 (cm^2)	E38M51-292	BW	12	3.44	0.06	314	370	418	12.0
DS	Dry mass stem (g)	P14M61-424	BW	14	3.46	0.04	3.3	3.4	4.2	13.1
DT	Total dry mass (g)	P14M61-424	BW	14	3.37	0.05	6.1	5.9	7.4	12.9
RL	RLGR (cm^2 cm^{-2} d^{-1})	P11M60-233	BW	10	3.10	0.1	0.102	0.100	0.105	10.9
LM	LMF (g g^{-1})	P14M50-225	JA	14	3.59	0.004	0.45	0.44	0.41	12.3
NL1	NRLV_39	E38M49-281	JA	2	3.17	0.02	4.3	4.7	5.3	11.1
NL2	NRLV_49	P21M50-258	JA	1	3.97	0.02	9.9	8.4	8.5	13.5
NL3	NRLV_60	P21M50-258	JA	1	6.57	0.02	18.2	14.2	14.4	21.4
BH	Branching height (cm)	P11M60-233	BW	10	4.07	0.02	48.9	50.6	40.2	14.1
BT1	Time till branching	P11M60-236	JA	5	3.61	0.04	49.6	55.7	55.4	10.9
BT2	Time till branching	P11M60-233	BW	10	5.16	0.003	55.9	56.1	49.0	16.0
NL4	NRLV on the main stem	P11M60-233	BW	10	3.90	0.02	12.64	12.82	10.46	13.4
IL	Internode length (cm)	P14M50-490	BW	14	3.06	0.04	3.47	4.25	4.24	11.0
LW1	L:W-ratio leaf	P14M47-474	JA	2	3.47	0.04	2.00	2.02	2.16	9.9
LW2	L:W-ratio leaf	P11M47-221	BW	16	3.94	0.02	2.17	2.06	1.99	10.9
FL	Fruit length (cm)	E32M48-142	BW	1	18.53	<0.001	12.4	10.9	8.4	52.5
FR	Fruit L:W-ratio	E32M48-142	BW	1	19.00	<0.001	3.9	3.3	2.2	52.9
FW	Fruit width (cm)	E32M48-142	BW	1	6.25	<0.001	3.2	3.5	3.9	22.2

a) Parent in which the AFLP fragment was amplified:; JA= Jatilaba; BW= Bruinsma Wonder

b) Genome wide LOD significance per trait

c) Average values for the different marker genotypes (homozygous for the for Jatilaba (JA) or Bruinsma Wonder (BW) allele or heterozygous (H))

d) Percentage of phenotypic variation explained by the QTL

RESULTS

Heritabilities of traits

Phenotypic characteristics related to plant growth and development were measured in a group of 150 F_2 plants and their parental varieties during a period of 68 days. During measurements, eight F_2 plants died or were damaged, hampering a normal growth and development. The remaining 142 F_2 plants were further analysed. The parental *C. annuum* varieties 'Jatilaba' and 'Bruinsma Wonder' exhibited large differences in phenotypic characteristics (Table 5.1). For most traits high heritabilities were found. Variation within 'Nassau F_1' was slightly higher than that of the open pollinated parental lines. Almost all traits for which significant QTLs were identified (Table 5.2) showed high heritabilities (Table 5.1).

Linkage map

Leaf material of plants propagated from cuttings of the original individual F_2 plants was used for DNA analysis, as harvesting leaves from the experimental plants themselves would affect the measurements. Hundred twenty-seven of the 142 F_2 plants produced viable cuttings. Analysis of 15 primer combinations on the 127 propagated F_2 plants together with the parental lines yielded 142 co-dominant markers. Five markers were not linked to other markers, and six were only linked in pairs at LOD >= 4; the remaining 131 markers were mapped into linkage groups. Figure 5.1 shows the linkage groups on which QTLs were identified. Our total genetic map consisted of 16 linkage groups while the haploid genome of *Capsicum* consists of 12 chromosomes. The map length was 817 cM.

QTL mapping

For a number of morphological and physiological traits, markers were found with significant QTL effects through MQM analysis (Table 5.2; Figure 5.1). One marker was identified with a highly significant effect on three fruit shape characteristics. The QTL region for this marker was identified on linkage group 1. For leaf length:width ratio, two markers were found on linkage groups 2 and

16 with significant QTL effects. Collectively they explained about 21% of the phenotypic variation for leaf length:width-ratio.

With respect to plant growth and development one QTL region was tentatively identified for RLGR on linkage group 10. The LOD score of this QTL was 3.10 (P = 0.10). For several plant morphological traits related to RLGR, markers were identified with significant QTL effects: stem dry mass (DS) and total dry mass (DT) were linked to the same marker on linkage group 14. This QTL accounted for 13% of the phenotypic variation for both traits. Furthermore a QTL region was identified for leaf area at 60 d.a.s on linkage group 12. A significant QTL for LMF, explaining 12% of the phenotypic variation for this trait, was found on linkage group 14.

Two QTLs for the number of leaves were identified. At 39 d.a.s., a QTL on linkage group 2 was found. Later during development (49 and 60 d.a.s.) the effect of this QTL disappeared and a QTL on linkage group 1 became important (Table 5.2). The effect of this second QTL became more pronounced in time: At 49 d.a.s. it accounted for 14% of the phenotypic variation whereas this increased to 21% at 60 d.a.s.

Finally, we studied three parameters with respect to branching of the main stem. Of these parameters, height of the branching point was positively correlated (R^2 = 0.52; P < 0.01) to the number of leaves on the main stem and the time till branching (R^2 = 0.17; P < 0.01); the last two were also positively correlated (R^2 = 0.28; P < 0.01). We found one QTL on linkage group 10 with an effect on all three traits, and a second QTL with an effect on time till branching on linkage group 5 (Table 5.2). Morphologically related to the branching traits is the internode length of the main stem. A QTL for this trait was identified on linkage group 14 (Table 5.2, Figure 5.1).

QTL validation

The effects of the QTLs identified in the F_2 population were validated in a set of 25 F_3 lines where eight F_3 plants represented the genotype of a single F_2 plant. F_3 lines were selected based on the homozygous presence of the Bruinsma Wonder or the Jatilaba allele of the QTL for RLGR, which also had an effect on several other traits. This specific QTL was chosen because RLGR is an important agronomic trait. Fruit shape traits were the only characteristics that

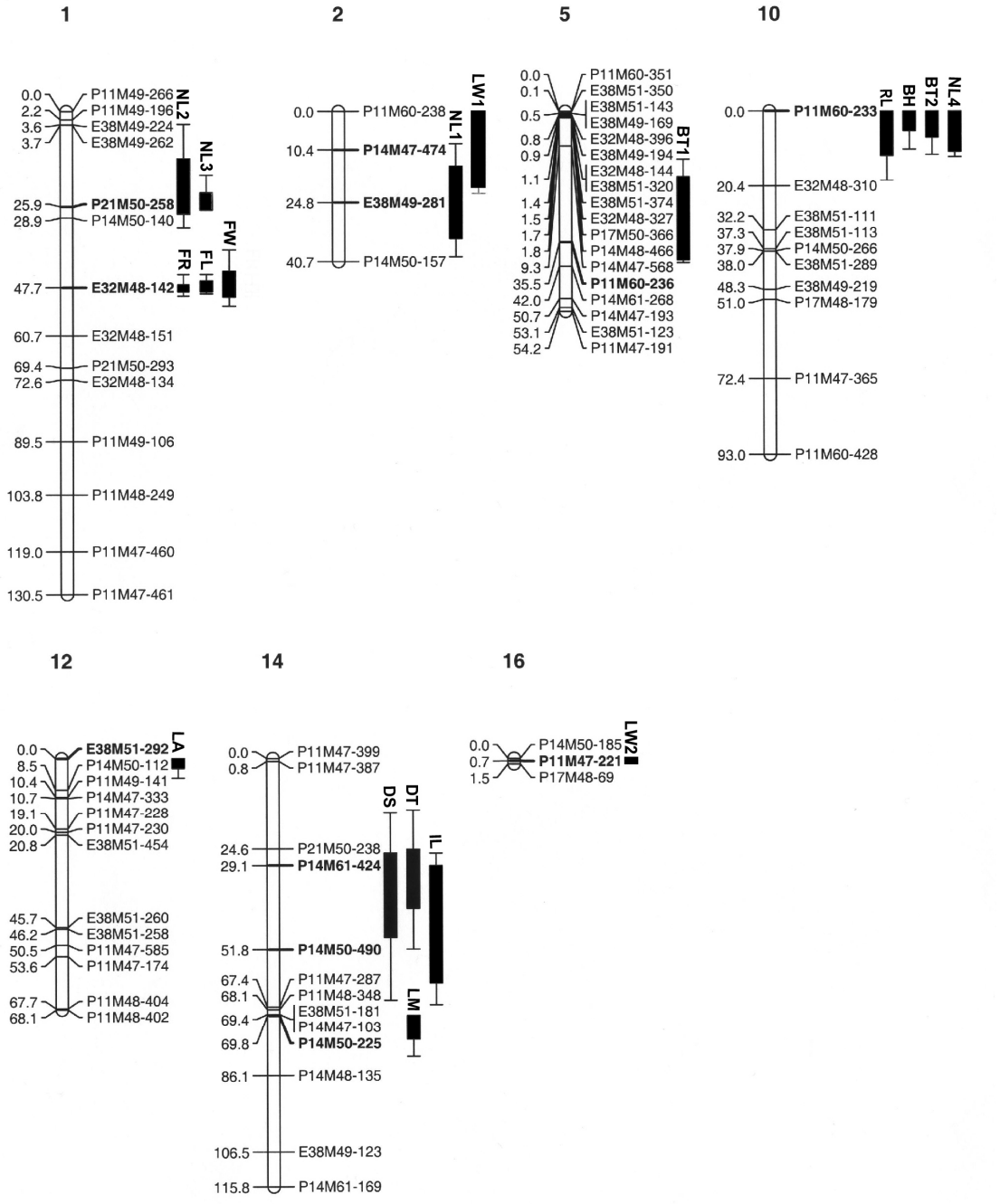


Figure 5.1: *Support intervals (1-LOD and 2-LOD) for growth-related trait QTLs identified in the F_2 '(Jatilaba x Bruinsma Wonder)' population. Numbers indicate the linkage groups, markers in bold indicate the markers with the highest LOD scores per QTL. Abbreviations of the QTLs are given in table 5.2. Genetic distances are given in cM*

were measured in the F_2 population but not in the F_3 lines. ANOVA showed significant year x genotype interaction ($P < 0.01$) between the parental lines grown in the F_2 and F_3 experiment for all measured traits except leaf length:width ratio.

For the traits measured both in the F_2 and F_3, a total of 15 QTL were identified in the F_2. For each QTL the F_2 population was divided into three genotypic groups: the two QTL homozygotes and the QTL heterozygote. For 7 of the 15 QTLs (DS, DT, RLGR, LMF, BH, NRLV_39 and NRLV_60) the F_3 means differed significantly in the same way as the corresponding F_2 means, confirming the QTL effects found in the F_2 generation (Figure 5.2). The effect of the weak QTL for leaf area at 60 d.a.s (Figure 5.2A), the QTL for number of leaves at 49 d.a.s. (Fig 5.2G) and of one of the two QTLs for Leaf L:W ratio (Fig 5.2N) could not be confirmed, although the relationships between the phenotypic data within the genotypic groups in the F_2 and F_3 experiment showed the same trend. For the other five QTLs: both QTLs for branching time (Figure 5.2J-K), the number of leaves on the main stem (Figure 5.2I), internode length (Figure 5.2M) and the second QTL for leaf length:width ratio (Figure. 5.2O), the results of the F_2 and F_3 experiments did not match.

The effect of the QTLs for dry mass of the stem (Figure 5.2B) and total dry mass (Figure 5.2C) were established in the F_3 lines even though dry mass was measured seven days earlier in the F_3 experiment than in the F_2 experiment. This shows that the effect of this QTL is conserved between generations over a range of development stages. The effect of the weak QTL for RLGR (Figure 5.2D) and of the QTL for branching height (Figure 5.2I), could be established in the F_3 lines for both homozygous genotypic groups, but no F_3 lines were available from heterozygous F_2 plants.

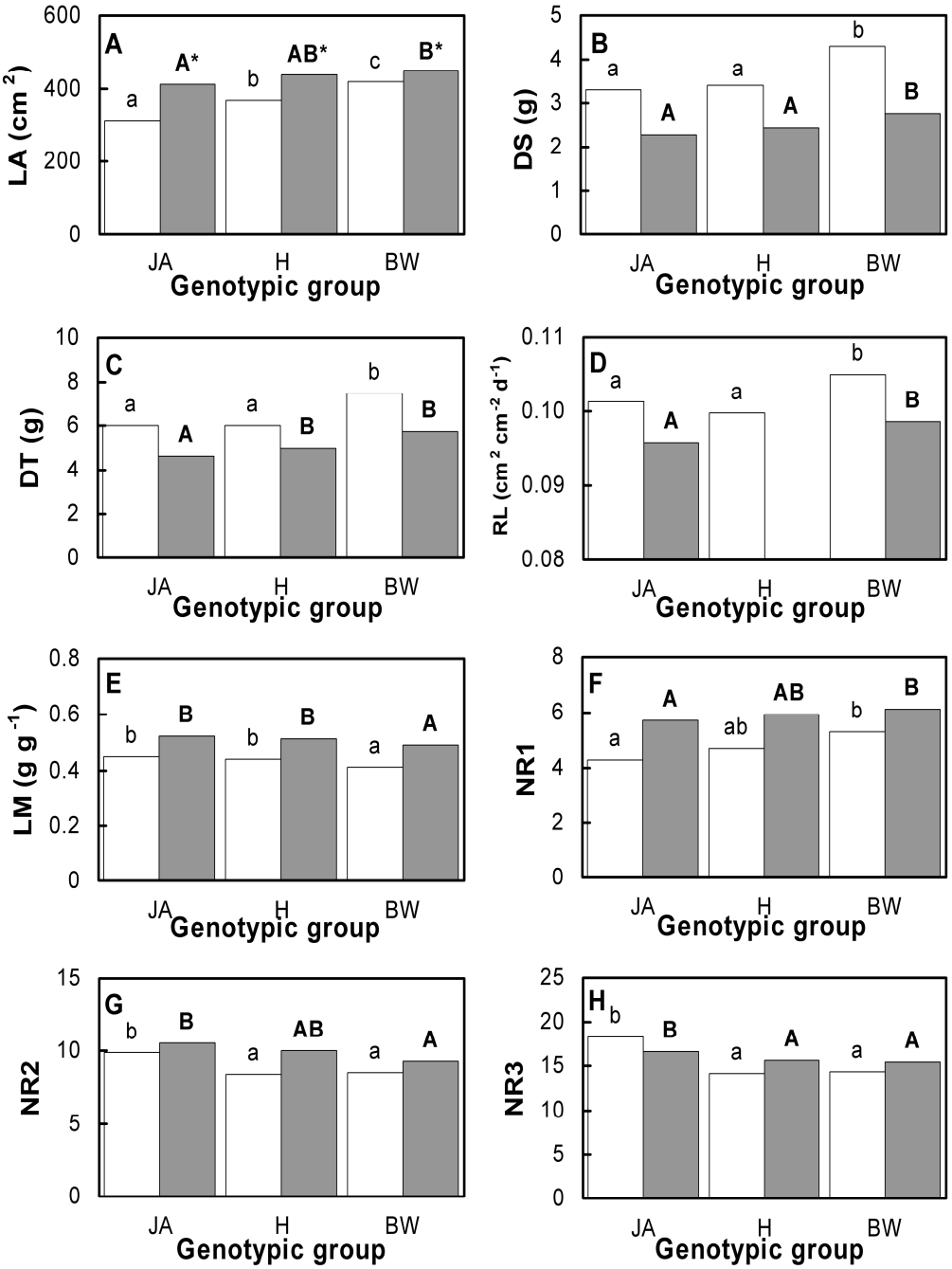
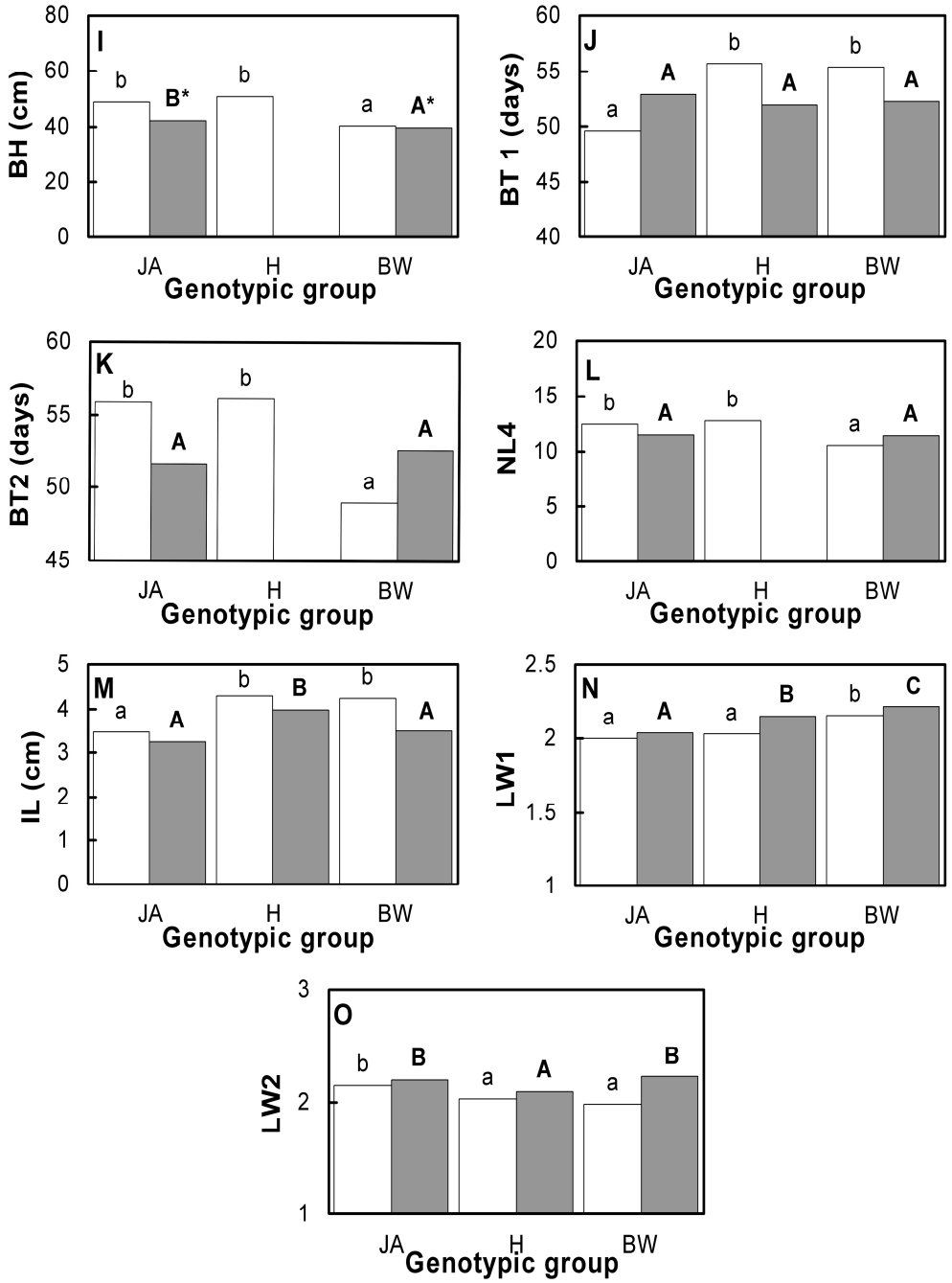


Figure 5.2: *Comparisons between the average values of measured traits for the different QTL genotypes (JA= homozygous Jatilaba allele; BW= homozygous Bruinsma Wonder allele, H= heterozygous) in the F_2 population (white bars) and the F_3 lines (grey bars) for (A) leaf area at 60 d.a.s.; (B) stem dry mass; (C) total dry mass; (D) relative leaf growth rate; (E) leaf mass fraction; number of leaves at (F) 39; (G) 49; or (H) 60 d.a.s.; (I) branching height; time till branching at QTLs (J) BT1 and*



(K) BT2; (L) number of leaves on the main stem; (M) internode length; and leaf length: width ratio at QTLs (N) LW1; and (O) LW2. Abbreviations of the traits are given in Table 5.2. For some traits, no data of heterozygous F_3 lines were available. Different letters indicate significant differences between the genotypic groups at P = 0.01 or P = 0.05 (*) in the F_2 population (normal script) or F_3 population (bold capitals)

DISCUSSION

Linkage map

The map that we constructed had a total length of 817 cM (Figure 5.1). This is considerably smaller than intra- and interspecific *Capsicum* maps published by Livingstone *et al.* (1999; 1245.7 cM), Kang *et al.* (2000; 2521 cM), Kang *et al.* (2001; 1320 cM), Lefebvre *et al.* (2002; 685 to 1668 cM), Ben Chaim *et al.* (2001b; 1740 cM), Lee *et al.* (2004; 1761.5 cM) and the integrated map of Paran *et al.* (2004; 1832 cM, http://www.plbr.cornell.edu/psi/peppers.html). The relatively short map presented in this study, together with the large number of linkage groups indicates that this map does not cover the whole genome.

Comparison of our linkage groups to the chromosomes on the map of Paran *et al.* (2004) showed that two of our linkage groups could be assigned to chromosomes. In the first place, our linkage group 3 had four markers in common with chromosome 11 of Paran *et al.* (2004). These markers were in the same order on both maps (E38M49-202 and E38/M49-F-202; P14M47-136 and P14/M47-F-137; E32M48-182 and E32/M48-F-183; E38M49-400 and E38/M49-F-400) indicating that our linkage group 3 corresponds to chromosome 11. Secondly, our linkage group 1 (Figure 5.1) showed a high degree of similarity with chromosome 3. Of the 5 common markers, three markers on chromosome 3 (P11/M49-F-106, E32/M48-F-135, E32/M48-F-152) were mapped in the same order as the P11M49-106, E32M48-134 and E32M48-151 markers on linkage group 1. Two markers (P11/M49-F-196 and E38/M49-F-224) were switched compared to the matching fragments on our own map but their distance was small on both maps (12 cM). This strongly indicates that our linkage group 1 corresponds to chromosome 3.

QTL mapping

The evidence that linkage group 1 corresponds with chromosome 3 was strengthened by the identification of a single QTL region for fruit shape traits on linkage group 1 (Table 5.2; Figure 5.1). On chromosome 3, the same region was identified as a major region for fruit shape traits in an intraspecific *C.*

annuum cross (Ben Chaim *et al.*, 2001b). Later this *fs3.1* QTL was identified in interspecific crosses as well (Ben Chaim *et al.*, 2003b; Rao *et al.*, 2003).

The effects of seven QTLs for agronomic important traits related to plant growth and development, which were identified in the F_2 population, could be established in the F_3 lines (Figure 5.2). This shows that, in contrast to the conclusions of Remington and Purugganan (2003) and of Kearsey and Farquhar (1998) and notwithstanding the genotype x year interactions found for most measured traits, a relatively small F_2 population can be of great value for studying growth and growth related QTLs provided that it is combined with confirming the detected QTLs in, for example, an F_3 generation.

The most important agronomic trait for which a QTL could be identified was RLGR (Figure 5.2D). RLGR is a measure for leaf area growth in time analogous to relative growth rate (Evans, 1972), which can be used as a measure for RGR in *Capsicum* (Chapter 4). RGR itself is a reliable predictor for dry mass development of plants during later development (De Swart *et al.*, 2006; Si and Thurling, 2001a). In other crops several authors also tried to identify QTLs for RLGR or RGR, with varying success. Yin *et al.* (1999b) could not identify a QTL for RLGR in *Hordeum vulgare* and Courtois *et al.* (2000) were unable to detect a QTL for RGR in rice. El-Lithy *et al.* (2004) on the other hand, identified QTLs for both RGR and RLGR in *Arabidopsis*. QTLs for RGR are were also found in *H. spontaneum* (Elberse *et al.*, 2004; Poorter *et al.*, 2005; Van Rijn, 2001), *Aegilops* (Ter Steege *et al.*, 2005) and *Salix* (Weih *et al.*, 2006). Van Rijn (2001) and Courtois *et al.* (2000) suggested that RGR is a trait which is controlled by several loci on the genome all having a small individual contribution, which are therefore difficult to detect. In our case, the identified QTL for RLGR in the F_2 population only explains 11 percent of the phenotypic variation for this trait (Table 5.2). In spite of this limited contribution, the QTL effect could be established in the F_3 lines, indicating that the QTL effect is stable over generations and years.

One of the traits related to RGR for which a QTL was identified and confirmed is leaf mass fraction (Table 5.2; Figure 2E). QTLs for this trait were also found by others (Elberse *et al.*, 2004; Poorter *et al.*, 2005; Ter Steege *et al.*, 2005). However, the value of LMF for purposes of breeding for low temperature tolerance in *Capsicum* is limited since LMF does not contribute

significantly to variation in RGR in *Capsicum* (De Swart *et al.*, 2006). Other morphological traits related to RGR for which QTLs were identified in the F_2 population and that were established in the F_3 lines were stem dry mass and total dry mass (Figure 5.2B-C) and the number of leaves at 39 d.a.s. and 60 d.a.s (Figure 5.2F, H). El-Lithy *et al.* (2004) also found QTLs for these traits in *Arabidopsis*.

In contrast to Ben Chaim *et al.* (2001b), we were unable to identify a QTL for total plant height in *Capsicum*, although a QTL for the height of the branching point was found (Table 5.2). We did find heterosis for plant height in *Capsicum* (Table 5.1), which was reported earlier by Ben Chaim and Paran (2000) and Meshram and Mukewar (1986). In other studies it was shown that QTLs for plant height within a particular year or environment were not applicable to other years (Yin *et al.*, 1999b) or locations (Hittalmani *et al.*, 2002). This shows the importance to test QTLs in multiple years and probably in more generations.

CONCLUSIONS

Our results illustrate that the effects of the individual loci with a relatively small contribution to a trait, are measurable in successive generations. Furthermore it can be concluded that a relatively small F_2 population can be of great value for studying growth and growth related QTLs provided that it is combined with confirming the detected QTLs in, for example, an F_3 generation. Finally our results indicate that there are perspectives for marker assisted breeding for growth and development in *Capsicum.*

Chapter 6

General discussion

Sweet pepper (*Capsicum annuum* L.) is an important greenhouse crop in the Netherlands. In 2005, the production area of sweet pepper in the Netherlands exceeded 1230 ha (CBS, 2006). *Capsicum* plants generally require relatively high temperatures to grow and produce fruit. As a consequence, the energy input needed to grow sweet pepper is very high. A simple approach to save energy is to lower the greenhouse temperature. To do so cultivars are needed that tolerate lowered temperatures without loss of yield. In this thesis the possibility to breed sweet pepper cultivars adapted to cooler growing conditions is studied. The relative difference in relative growth rate (RGR) between plants grown at different temperature conditions was used as a measure for adaptation to low temperature (Chapter 1). Here the conclusions derived from Chapters 2 through 5 will be discussed and integrated. Also, the usefulness of the results for practical application will be discussed. The discussion ends with recommendations for future investigations and an overview of the main conclusions.

GENERAL OVERVIEW

Chapter 2 describes a method to estimate leaf area from non-destructive measurements in *C. annuum*. This method is used in Chapter 5 for repeated non-destructive measurements of leaf area on genetically unique F_2 and F_3 plants. As described in Chapter 2, leaf area could be predicted from the product of leaf length and width ($\alpha \cdot L \cdot W$); by the addition of both linear and quadratic leaf width terms the model ($Area = 0.61906 \cdot L \cdot W + 0.2060 \cdot W^2 - 0.5142 \cdot W$) was shown to become independent of plant age and accession and could be used for leaf area estimation during the whole vegetative phase as well as at the start of the generative growth phase of *Capsicum*. It was demonstrated that this model predicts total plant leaf area quite accurately, even when the length and width of only 25% of the leaves were measured.

The influence of lowered, sub-optimal temperature on growth, growth related traits and plant morphology was studied in both a group of wild and cultivated *Capsicum* accessions (Chapter 3) as well as in a group of sweet

pepper cultivars (Chapter 4). The variation in adaptation to lowered temperatures in both groups was subsequently compared. The aim of Chapter 3 was to identify the variation in RGR and its underlying physiological and morphological traits in a group of wild and cultivated *Capsicum* accessions of four *Capsicum* species at two contrasting temperatures. To this end a growth analysis was performed on young plants grown at two temperature regimes (21.1°/18.7°C and 17.3°/14.7°C; day/night). Based on the relative difference in RGR between the *Capsicum* accessions at different temperatures, some *Capsicum* accessions were shown to be better adapted to lowered temperatures than others, indicating an opportunity to breed for lowered temperature tolerance in *Capsicum*. Under both temperature regimes net assimilation rate (NAR) proved to be the most important factor explaining the variation in RGR among the *Capsicum* accessions. The variation in the reduction of RGR under lowered temperatures was due to changes in both NAR and leaf area ratio (LAR).

To identify which traits can be used to simplify the selection for RGR in breeding programmes, the relationship between RGR and a number of physiological and morphological plant traits (relative leaf growth rate (RLGR), leaf, stem and total fresh and dry mass, plant height, leaf area, number of leaves) was studied both in a number of wild and cultivated *Capsicum* accessions and a number of sweet pepper cultivars (Chapter 4). RLGR was strongly correlated with RGR and could therefore serve as a reliable predictor of RGR. Together with the non-destructive measurement of leaf area described in Chapter 2, this greatly simplifies the estimation of RGR in *Capsicum* for practical purposes. The relations between RGR and other plant traits could only demonstrated when a considerable amount of variation for these traits was present in the experimental group of plants. Variation for lowered temperature tolerance within the group of sweet pepper cultivars was limited, indicating that sweet pepper breeders need to exploit the genetic variation in wild relative species in order to develop cultivars with increased energy efficiency.

The possibility to use this physiological and morphological information on *Capsicum* in breeding programmes for lowered temperature tolerance is described in Chapter 5. The inheritance of RLGR and a number of

physiological and morphological plants traits were examined in an intraspecific *C. annuum* F_2 population. A total number of 15 QTLs were identified in the F_2 population; the effect of seven QTLs related to plant growth and development (e.g. RLGR, leaf mass fraction (LMF), dry mass, number of leaves) was validated in a group of 25 selected F_3 lines. The results illustrate that the effects of the individual loci, each with a relatively small contribution to a trait, are measurable in successive generations, indicating the possibility for marker assisted breeding in *Capsicum*.

GROWTH AND DEVELOPMENT, PHYSIOLOGY AND MORPHOLOGY

Vegetative growth phase versus generative growth phase

In the introduction we assumed that plants with a high growth rate in their vegetative phase (fast growers) also exhibited fast growth during their generative phase (Chapter 1). Within the group of wild and cultivated *Capsicum* accessions, RGR at the moment of branching was positively correlated to dry mass at 63 (standard temperatures) and 98 (lowered temperatures) days after sowing (Chapter 3). These results compare to results found in *Brassica* (Si and Thurling, 2001a) and wheat (Karimi and Siddeque, 1991) where positive correlations between RGR during the vegetative growth phase on the one hand and dry mass at anthesis or biomass production and grain yield on the other hand were found. Si and Thurling (2001b) further discussed the possibility to identify genotypes with vigorous pre-anthesis growth and early flowering since pre-anthesis dry matter production under low temperature could be increased without delaying time to flowering. In *Capsicum*, Claphan and Marsh (1987) found that increased vegetative growth resulted in an increase in both total plant dry mass and fruit production. In cucumber, young plants of accessions which performed relatively better at sub-optimal temperatures compared to others, also exhibited faster growth at a mature stage (Bulder *et al.*, 1987). Owona (2005) further discussed that tomato lines which showed a high RGR in the early phase of plant development and maintained a relatively high growth rate during later plant development were the most promising lines to select for with respect to crop

performance. Furthermore, early vigour or rapid vegetative growth in rice (Jones *et al.*, 1997) and wheat (Botwright *et al.*, 2002; Whan *et al.*, 1991) were associated with high biomass production and increased yield. In olive, pre-selection of olive seedlings for earliness of first flowering is possible, based on vegetative characteristics assessed very early in their development (Pritsa *et al.*, 2003). Generally, vegetative growth seems to be linked to biomass production in the generative phase. Therefore, selection for rapid growth in the vegetative growth phase of *Capsicum* may indeed be an efficient method for the selection of plants with good growth and dry mass production in the generative phase. The allocation to different plant organs (roots, stems, leaves and fruits), however, changes during vegetative growth in *Capsicum* (Claphan and Marsh, 1987). Biomass allocation, and hence fruit yield can not be predicted based on early growth. These also depend on other factors as, for example, row arrangements (Kahn and Leskovar, 2006), plant density (Heuvelink and Marcelis, 1996; Jovicich *et al.*, 2004; Marcelis *et al.*, 2004; Ortega *et al.*, 2004), irrigation regimes (Sezen *et al.*, 2006) and fruit and leaf pruning (Heuvelink and Marcelis, 1996; Marcelis *et al.*, 2004).

RGR and growth related traits

RGR is an important determinant of plant growth and development. RGR can be divided into two components, i.e. NAR and LAR. LAR can further be divided into specific leaf area (SLA) and LMF (Evans, 1972). We showed that in *Capsicum*, NAR is the most important growth related trait explaining variation among genotypes in RGR (Chapter 3). Although it is commonly observed that LAR, and more specifically SLA, is the most important factor to explain variation in RGR between species (reviewed by Poorter, 1989a; Poorter and Van Der Werf, 1998), studies comparing species belonging to the same genus (Atkin *et al.*, 1996; Van der Ploeg *et al.*, 2005; Villar *et al.*, 1998), accessions of the same species (Biere, 1996; Owona, 2005; Si and Thurling, 2001a; Verhoeven *et al.*, 2004) and even closely-related grass species (Garnier, 1992; Reiser *et al.*, 2002) lead to the same conclusions as ours in *Capsicum* (Chapter 3). Meerts and Garnier (1996) on the other hand, found that in a group of *Polygonum aviculare* genotypes, NAR negatively correlated with RGR. Finally, in keeping with our conclusions in Chapter 3, a recent meta-

analysis of woody and herbaceous species (Shipley, 2006) also led to the conclusion that, in general, NAR was the best predictor of RGR.

Changes in RGR due to temperature are often attributed to changes in LAR and not to changes in NAR. This was for example found in greenhouse crops as tomato (Heuvelink, 1989; Nieuwhof *et al.*, 1993), cucumber (Den Nijs and Smeets, 1987) and sweet pepper (Nilwik, 1981b). In Chapter 3 however, it was shown that the variation in the reduction of RGR under lowered temperatures in *Capsicum* was due to changes in both NAR and LAR. In keeping with these findings, *Capsicum* accessions with the smallest reduction in RGR under lowered temperatures also showed the smallest decrease in either NAR or LAR or in both components (Figure 3.4A-C). In other accessions, the larger reduction in RGR was due to a combination of large reductions in both NAR and LAR.

One might argue that the ideal lowered temperature adapted sweet pepper variety should have both a high NAR and high LAR under lowered temperatures. Stehli *et al.* (1999) suggested that the combination of a relative high NAR and LAR under cooler regimes could be used to produce cold-adapted maize varieties with a superior late growth and production. In chrysanthemum, a higher RGR could be achieved by combining a high partitioning toward the leaves (high LWR), thin leaves (high SLA), and a high NAR (Van der Ploeg *et al.*, 2007). Nieuwhof *et al.* (1993) proposed to cross tomato genotypes with either a high NAR or a high LAR in order to increase RGR. Smeets and Garretsen (1986) on the other hand questioned if a cross between a tomato line with high RGR and NAR and a line with high RGR and LAR, would yield an F_2 plant which both a high NAR and LAR because of the physiologically imposed negative correlation between LAR and NAR. Due to a negative correlation between NAR and LAR, an increase in NAR will generally decrease LAR, and this in turn will have a negative effect on RGR, thus masking the correlation between NAR and RGR (Poorter and Remkes, 1990).

In a group of 10 *Capsicum* accessions this negative correlation between NAR and LAR was found under both temperature regimes (Figure 3.3). Nevertheless, one accession was found that combined relatively high NAR and LAR under lowered temperatures, which reduced also the decrease in RGR under lowered temperatures compared to other *Capsicum* accessions.

Therefore, in spite of the trade-off between NAR and LAR in many accessions the opportunity exists to combine a high NAR and high LAR in a single genotype resulting in an increased RGR.

The value of RGR for breeders

Growth analysis is a good tool to study the response of plants to varying environmental conditions (Grime and Hunt, 1975). Especially in ecophysiological studies, growth analysis has proven to be very valuable because it provides a high level of understanding of RGR and its underlying physiological and morphological components in experiments with varying external factors. Although RGR is an important plant characteristic, its value for breeding purposes is not yet clear. Much of the physiological research is not as yet being used for actual breeding purposes other than making suggestions about possible approaches to selection. Integration of physiology into breeding programs can be achieved by identifying traits for direct or indirect selection as an adjunct to criteria already used (Jackson *et al.*, 1996).

In this thesis RGR was specifically chosen for its physiological value. RGR was shown to be an important plant trait to determine the presence of low temperature tolerance in sweet pepper (Chapter 3). Similar to our findings, Sparnaaij *et al.* (1996) concluded that in breeding for dry matter production in the vegetative phase of carnation, an analysis of RGR and its components provides more relevant information than that of dry mass only. Furthermore, plant growth analysis in terms of RGR and related growth traits leads to new applications in breeding practice: in wheat RGR can be used for selection of rapid juvenile growth and hence for earlier canopy closure and therefore higher yield in monoculture (Spitters and Kramer, 1986). Whan *et al.* (1991) argued that it is important that the value of any physiological character is assessed in breeding populations before it is used by breeders. Although complex physiological traits such as RGR are difficult and expensive to assess (Jackson et al., 1996), RGR provides important information about general plant growth and development. By identifying physiological and morphological traits that are related to RGR but are easier to assess than RGR itself, indirect selection for RGR may become feasible in practical breeding.

Relative Leaf Growth Rate

One of the candidate traits that can be used to select indirectly for RGR is the relative leaf growth rate (RLGR). High correlations between RGR and RLGR were found earlier in *Brassica* (Paul, 1992), maize (Verheul *et al.*, 1996) and within a group of nine different species (Potter and Jones, 1977). In maize, RLGR was even used as a selection criterion to increase grain yield in a successful breeding program (Nevado and Cross, 1990). In our experiments with *Capsicum* RGR was strongly related to RLGR even in experimental groups where variation for RGR was only limited (Figure 4.2 and 4.3), giving the first indication that RLGR can adequately replace RGR in *Capsicum*.

An obvious advantage of using RLGR over RGR is that it does not require destructive measurements. Leaf area can be measured either by destructive or non-destructive measurements. In Chapter 2 a model is presented to estimate leaf area based solely on measurements of leaf length and leaf width (Area = $0.61906 \bullet L \bullet W + 0.2060 \bullet W^2 - 0.5142 \bullet W$). Thus, total plant leaf area can be determined repeatedly on single plants without harvesting and sacrificing them. RLGR can be calculated from these leaf area data. The value of this method is shown in Chapter 5 where a large number of genetically unique F_2 and F_3 plants could be measured several times. These findings together with the identification and validation of a QTL for RLGR (Chapter 5, see also below), makes RLGR a valuable, easy-to-measure trait that can be used as an indirect measure for RGR.

LOWERED TEMPERATURE TOLERANCE IN *CAPSICUM*

The possibility to breed for lowered temperature tolerance is discussed in Chapter 3. A group of ten wild and cultivated *Capsicum* accessions of four *Capsicum* species was grown at standard greenhouse temperatures and at lowered temperatures (four degrees below standard temperature). The wild *Capsicum* accessions used in this chapter were specifically chosen based on average daily temperatures in their natural habitats (Table 3.1). Within this group a considerable amount of variation in adaptation to lowered

temperatures was found. The relative difference in RGR between plants grown under various temperature regimes ranged from (minus) 20% to 49% (Figure 3.4A). Within the group of sweet pepper cultivars the range in RGR change was less, but the average change was larger: a reduction of 43% to 52% (Chapter 4). Especially one *C. pubescens* accession, known to be adapted to cooler growing conditions (Bosland, 1996), proved to adapt well to lowered temperatures, but also *C. baccatum* 'PI 585248' showed a much lower decrease in RGR (37%) under lowered temperatures than was found in sweet pepper. Another indication of the presence of cold tolerance in this species is that some accessions of *C. baccatum* are known to be able to germinate at temperatures between 10° and 13°C (Randle and Honma, 1980).

Within the limited plant material described in this thesis, most variation to lowered temperature tolerance seems to be present in *C. pubescens*. This species, however, is difficult to use in breeding programs for sweet pepper improvement since it will not directly hybridise with *C. annuum* (Smith *et al.*, 1987). Although various methods have been mentioned to obtain crosses between *C. pubescens* and *C. annuum* (Molchova and Michailova, 1982; Pickersgill, 1988; Zijlstra *et al.*, 1991) little or no information is available about successful hybridisations or bridge crosses. *C. baccatum* on the other hand will hybridise with *C. annuum* (Pickersgill, 1971, 1988; Smith *et al.*, 1987). *C. baccatum* 'PI 585248' was also much better adapted to lowered temperatures than the sweet pepper cultivars measured in Chapter 4. Based on the variation for lowered temperature tolerance found in the limited plant material studied in this thesis, it might be expected that more variation is present within the whole *Capsicum* genus. As in tomato (Van der Ploeg and Heuvelink, 2005), a further exploitation of the genetic variation for lowered temperature tolerance in wild relative *Capsicum* species is recommendable in order to breed cultivars with increased energy efficiency.

GENETICS

RLGR and growth related traits

The genetics of RLGR and a number of physiological and morphological plant traits were examined in an intraspecific F_2 population and a total number of 15 QTLs were identified (Chapter 5). The most important agronomic trait for which a QTL could be identified and validated was RLGR, despite the low heritability of this trait (Table 5.1). According to Culley *et al.* (2005), low heritability of some ecophysiological traits may reflect low additive genetic variability or high phenotypic plasticity in these traits. As discussed above, RLGR is a valuable, easy-to-measure trait that can be used as a reliable predictor of RGR in *Capsicum* both under standard and lowered temperatures (Chapter 4). In contrast to studies in barley (Yin *et al.*, 1999a) and maize (Hund *et al.*, 2005), no QTLs for SLA could be identified in *Capsicum* (Chapter 5). Although one QTL for LMF in Capsicum was identified and verified, LMF is not correlated to RGR (Fig 3.2D) and is therefore of no use for breeding for lowered temperature tolerance. In wild barley QTLs for the other growth related traits, NAR (Poorter *et al.*, 2005) and LAR (Elberse *et al.*, 2004; Poorter *et al.*, 2005) were identified. Earlier we discussed that both NAR and LAR contributed to the adaptation of *Capsicum* plants to lowered temperatures. Unfortunately, in this study, it was impossible to measure these important traits in the F_2 population. An F_2 population consists of genetically unique plants which can only be harvested once. Assessment of NAR requires repeated destructive measurements of dry mass which is impossible with unique, single plants. LAR could not be calculated because the final leaf area and final dry mass were not measured on the same day.

QTLs for easily measurable morphological traits

Morphological plant traits for which QTLs were determined were leaf area at 60 d.a.s., stem and total dry mass at final harvest and the number of leaves at 39, 49 and 60 d.a.s. (Table 5.2). Plant dry mass (leaf, stem and total), leaf area and number of leaves were all correlated to RGR from 35 d.a.s. onwards (Table 4.2) in a group of wild and cultivated *Capsicum* accessions where

considerable variation in RGR was present. The parental lines of the F_2 population, 'Jatilaba' and 'Bruinsma Wonder', were chosen because of their large differences in phenotypic characteristics among which RGR (Figure 4.2) and RLGR (Table 5.1). The QTLs for leaf area at 60 d.a.s., stem and total dry mass at final harvest and the number of leaves at 39, 49 and 60 d.a.s. are therefore very interesting in relation to RGR and growth and development of *Capsicum* plants. Although both parental lines are not promising with respect to lowered temperature tolerance, the QTLs characterised in this F_2 population and successive inbred lines, theoretically should have the potential to be used in breeding programs. In populations derived from other *Capsicum* crosses, QTLs for these and other traits have to be determined again. But at least it has been shown that QTLs for growth physiological traits can be found in *Capsicum*. Five other morphological traits for which QTLs were identified were branching height, time till branching (two QTLs), number of leaves on the main stem and internode length (Table 5.1). In the sweet pepper cultivars studied in Chapter 4 these traits were not correlated to RGR and therefore most likely they are only of limited value with respect to plant growth, although they might be more relevant with respect to plant development.

Influence of temperature on QTLs

The QTLs for growth and growth related traits were studied at standard temperatures. Environmental temperature is known to have an effect on the expression of QTLs for growth and development. In lettuce for example, QTLs for seed-related traits such as germination and seedling vigour were temperature dependent (Argyris *et al.*, 2005). Furthermore, QTLs for seedling vigour in rice appeared to be temperature specific (Zhang *et al.*, 2005). In maize, QTLs for SLA were temperature dependent (Hund *et al.*, 2005). Therefore, the QTLs for growth, growth related traits and morphology identified under standard temperatures in *Capsicum* (Chapter 5) are not necessarily effective under lowered temperatures. However, high correlations between morphological traits of plants grown under different temperatures were found (Table 4.1) and the RLGR of accessions grown under standard temperatures was positively correlated to RLGR of the same accessions under lowered temperatures (Chapter 4). Relating both findings it is conceivable that at least

some of the QTLs found under standard temperatures are likely to be effective under lowered temperatures as well.

PRACTICAL APPLICATIONS

The main objective of this thesis was to determine the possibilities to breed lowered temperature tolerant, and thus energy efficient, sweet pepper cultivars. This first required an understanding of the degree of low temperature tolerance that can be expected to be present in the genus *Capsicum* and, for breeding purposes, insight in how this knowledge can actually be incorporated into breeding programs. Temperature tolerance will never be the only characteristic that breeders consider important when developing new sweet pepper cultivars. With respect to consumers for example, it is important to know what kind of sweet pepper they prefer. Interestingly, Pet (1985) found that the fruits of some sweet pepper cultivars which were adapted to lowered temperatures were not appreciated by Dutch consumers and thus were not cultivated in the Netherlands. Thus breeders would have to break any negative association between taste and adaptedness to lowered growing temperatures. With respect to growers, breeders also have to consider the effect of lowered temperature on yield, susceptibility to diseases and greenhouse conditions such as relative humidity.

In this thesis we were only able to focus on lowered temperature tolerance in the vegetative phase of *Capsicum*. This has led to a greater understanding of early *Capsicum* growth and possible future applications. One important finding for practical application is the observation that variation for lowered temperature tolerance is present in *Capsicum* (Chapter 3 and 4) which is the first requirement to start a breeding program for sweet pepper that is adapted to cooler growing conditions. We also showed how to screen for this variation (Chapter 3 and 4) by using the relative decrease in RGR as a measure for lowered temperature tolerance. RLGR, as a predictor for RGR itself, is a valuable trait that may indicate adaptedness to lowered temperatures in a wide collection of exotic *Capsicum* accessions. RLGR can easily be determined using the model for non-destructive leaf area estimation

(Chapter 2) which is a good example of results of scientific research that can directly be brought to practice. Last but not least, a genetic analysis within *C. annuum* showed that it is possible to identify and validate QTLs for growth and growth related traits, proving that marker assisted selection for growth and development is possible in *Capsicum*. There is still a long way to go before lowered temperature tolerant sweet peppers can actually be produced. Knowledge and tools developed in this thesis may serve as a starting point for breeding programs for lowered temperature tolerance in sweet pepper.

Recommendations for future research

The number of accessions measured in Chapter 3 was limited and encompasses only a small part of the genetic variation available in *Capsicum*. The National Germplasm System (USA) for instance, has a collection of 3201 *C. annuum* accessions, 344 *C. baccatum* accessions and 78 *C. pubescens* accessions. In the Netherlands, the Centre for Genetic Resources (Wageningen, The Netherlands) accommodates a collection of 978 *Capsicum* accessions including 758 *C. annuum* accessions, 38 *C. baccatum* accessions and 6 *C. pubescens* accessions. Furthermore, the Botanical and Experimental Garden of the Radboud University (Nijmegen, The Netherlands) maintains a Solanaceae collection including 129 *Capsicum* accessions. Based on the variation for lowered temperature tolerance found in our small sample from the genus, it seems very likely that much more sources of tolerance to lowered temperature are present in the genus *Capsicum*, increasing the possibilities to breed for lowered temperature tolerance in sweet pepper.

A more thorough and extensive search for candidate accessions is now easier, based on the knowledge described in this thesis. I would therefore recommend that growth studies are expanded to more wild *Capsicum* accessions selected based on the temperature of their natural habitat and hence their expected adaptation to lowered temperatures. By combining a limited number of destructive measurements over time with a large number of non-destructive measurements of leaf area over time, space and time can be saved in screening large groups of accessions.

The further development of promising accessions with respect to lowered temperature tolerance should be monitored in the generative phase.

This would make it possible to directly link early growth of *Capsicum* to generative growth and yield. Furthermore, this would provide insight in the performance of lowered temperature tolerant *Capsicum* accessions under normal summer greenhouse temperatures.

The combination of vegetative and generative growth data makes better selection for breeding material possible. Combining this approach with genetic fingerprinting and association mapping, it could be used to assess both genetic and plant physiological diversity of a large number of *Capsicum* accessions easily. In this way both the variation in adaptation to lowered temperatures and the genetic heterogeneity of a germplasm collection and single *Capsicum* accessions would be revealed.

MAIN CONCLUSIONS

The work described in this thesis can be considered a quest to determine the possibilities to breed for lowered temperature tolerant, energy efficient sweet pepper cultivars. After several years of work the following conclusions were arrived at:

- Leaf area of *Capsicum* accessions can be estimated accurately and independent of leaf or plant age on basis of measurements of leaf length and leaf width.
- Within the genus *Capsicum* a considerable amount of variation in adaptation to lowered temperatures is present.
- This variation exceeds the variation for lowered temperature tolerance in currently grown sweet pepper cultivars, indicating that breeders should exploit the genetic variation in wild relative species in order to improve adaptedness to cooler growing conditions.
- In *Capsicum* the reduction of RGR under lowered temperatures is due to changes in both NAR and LAR.
- The ideal lowered temperature adapted sweet pepper variety has both a high NAR and high LAR under lowered temperatures
- NAR is the most important factor explaining variation in RGR between the different *Capsicum* accessions, independent of temperature treatment.

- RLGR is a valuable, easy-to-measure trait that can replace RGR and thus be used for determining variation for lowered temperature tolerance in *Capsicum*.
- Marker assisted selection can be used to breed for growth and development in *Capsicum*.

References

AJAYI, N. O. (1990). Rapid determination of leaf area in ovate vegetable leaves by linear measurements. *Journal of Horticultural Science & Biotechnology*, **65**, 1-5.

ANCHONDO, J. A., WALL, M. M., GUTSCHICK, V. P. and SMITH, D. W. (2002). Growth and yield of iron-deficient chile peppers is sand culture. *Journal of the American Society for Horticultural Science*, **127**, 205-210.

ANDAYA, V. C. and MACKILL, D. J. (2003). Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. *Journal of Experimental Botany*, **54**, 2579-2585.

ANONYMOUS. (1995). Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy: The Asian Vegetable Research and Development Center, Taipei, Taiwan, and the Centro Agronómico Tropical de Inverstigación y Enseñanza, Turrialba, Costa Rica. ISBN 92-9043-216-0.

ANONYMOUS. (1997). *Convernant glastuinbouw en milieu 1995 - 2010 met integrale milieu taakstelling (IMT)*. 39 pp.

ANONYMOUS. (1998). *Kyoto protocol to the United Nations framework convention on climate chance*. United Nations, 21 pp.

ANONYMOUS. (2004). *Evaluatie Besluit Glastuinbouw 2003*. LTO Groeiservice, Den Haag. 241 pp.

ARGYRIS, J., TRUCO, M. J., OCHOA, O., KNAPP, S. J., STILL, D. W., LENSSEN, G. M., SCHUT, J. W., MICHELMORE, R. W. and BRADFORD, K. J. (2005). Quantitative trait loci associated with seed and seedling traits in Lactuca. *Theoretical and Applied Genetics*, **111**, 1365-1376.

ATKIN, O. K., BOTMAN, B. and LAMBERS, H. (1996). The causes of inherently slow growth in alpine plants: An analysis based on the underlying carbon economies of alpine and lowland *Poa* species. *Functional Ecology*, **10**, 698-707.

ATKIN, O. K., LOVEYS, B. R., ATKINSON, L. J. and PONS, T. C. (2006). Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Experimental Botany*, **57**, 267-281.

BAENZIGER, P. S., RUSSELL, W. K., GRAEF, G. L. and CAMPBELL, B. T. (2006). Improving lives: 50 years of crop breeding, genetics, and cytology (C-1). *Crop Science*, **46**, 2230-2244.

BAKKER, J. C. (1989). The effect of air humidity on growth and fruit production of sweet pepper (*Capsicum annuum* L.). *Journal of Horticultural Science & Biotechnology*, **64**, 41-46.

BAKKER, J. C. and VAN UFFELEN, J. A. M. (1988). The effect of diurnal temperature regimes on growth and yield of glasshouse sweet pepper. *Netherlands Journal of Agricultural Sciences*, **36**, 201-208.

BANGE, M. P., HAMMER, G. L., MILROY, S. P. and RICKERT, K. G. (2000). Sunflower, improving estimates of individual leaf area of sunflower. *Agronomy Journal*, **92**, 761-765.

BARAL, J. B. and BOSLAND, P. W. (2004). Unravelling the species dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): a multiple evidence approach using morphology, molecular analysis, and sexual compatibility. *Journal of the American Society for Horticultural Science*, **129**, 826-832.

BEADLE, C. L., LONG, S. P., IMBAMBA, S. K., HALL, D. O. and OLEMBO, R. J. (1985). *Photosynthesis in relation to plant production in terrestrial environments*. Tycooly publishing limited, Oxford, UK. 156 pp.

BEDNARZ, C. W. and VAN IERSEL, M. W. (2001). Temperature response of whole-plant CO_2 exchange rates of four upland cotton cultivars differing in leaf shape and leaf pubescence. *Communications in Soil Science and Plant Analysis*, **32**, 2485-2501.

BEN CHAIM, A., BOROVSKY, Y., DE JONG, W. and PARAN, I. (2003a). Linkage of the locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. *Theoretical and Applied Genetics*, **106**, 889-894.

BEN CHAIM, A., BOROVSKY, Y., RAO, G. U., TANYOLAC, B. and PARAN, I. (2003b). fs3.1: major fruit shape QTL conserved in *Capsicum*. *Genome*, **46**, 1-9.

BEN CHAIM, A., GRUBE, R. C., LAPIDOT, M., JAHN, M. and PARAN, I. (2001a). Identification of qualitative trait loci associated with resistance to cucumber mosaic virus in *Capsicum annuum*. *Theoretical and Applied Genetics*, **102**, 1213-1220.

BEN CHAIM, A. and PARAN, I. (2000). Genetic analysis of quantitative traits in pepper (*Capsicum annuum*). *Journal of the American Society for Horticultural Science*, **125**, 66-70.

BEN CHAIM, A., PARAN, I., GRUBE, R. C., JAHN, M., VAN WIJK, R. and PELEMAN, J. (2001b). QTL mapping of fruit-related traits in pepper (*Capsicum annuum*). *Theoretical and Applied Genetics*, **102**, 1016-1028.

BHATT, R. M. and SRINIVASA ROA, N. K. (1993). Response of bell-pepper (*Capsicum annuum* L.) photosynthesis, growth and flower and fruit setting to night temperature. *Photosynthetica*, **28**, 127-132.

BIERE, A. (1996). Intra-specific variation in relative growth rate: Impact on competitive ability and performance of *Lychnis flos-cuculi* in habitats differing in soil fertility. *Plant and Soil*, **182**, 313-327.

BLUM, E., MAZOUREK, M., O'CONNELL, M., CURRY, J., THORUP, T., LIU, K., JAHN, M. and PARAN, I. (2003). Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum*. *Theoretical and Applied Genetics*, **108**, 79-86.

BOOTE, K. J. and SINCLAIR, T. R. (2006). Crop Physiology: Significant Discoveries and Our Changing Perspective on Research. *Crop Science*, **46**, 2270-2277.

BOSLAND, P. W. (1992). Chiles: A diverse crop. *HortTechnology*, **2**, 6-10.

BOSLAND, P. W. (1994). Chiles: history, cultivation, and uses. In: *Spices, Herbs, and Edible Fungi*. (Charalambous, G., Eds). Elsevier Publishers, New York, USA. 347-366.

BOSLAND, P. W. (1996). *Capsicums*: innovative uses of an ancient crop. In: *Progress in New Crops*. (Janick, J., Eds). ASHS Press, Arlington, VA, USA. 479-487.

BOSLAND, P. W. and VOTAVA, E. J. (2000). *Peppers: Vegetable and Spice Capsicums*. CABI Publishing, New York, USA. 250 pp.

BOT, G. P. A. (2001). Developments in indoor sustainable plant production with emphasis on energy saving. *Computers and electronics in agriculture*, **30**, 151-165.

BOTWRIGHT, T. L., CONDON, A. G., REBETZKE, G. J. and RICHARDS, R. A. (2002). Field evaluation of early vigour for genetic improvement of grain yield in wheat. *Australian Journal of Agricultural Research*, **53**, 1137-1145.

BRIGGS, F. N. and KNOWLES, P. F. (1967). *Introduction to plant breeding*. Reinhold Publishing Corporation, New York, London, Amsterdam. pp.

BRUGGINK, G. (1987). Influence of light on the growth of young tomato, cucumber and sweet pepper plants in the greenhouse: Calculating the effect of differences in light integral. *Scientia Horticulturae*, **31**, 175-183.

BRUGGINK, G. and HEUVELINK, E. (1987). Influence of light on the growth of young tomato, cucumber and sweet pepper plants in the greenhouse: effects on the Relative Growth Rate, Net Assimilation Rate and Leaf Area Ratio. *Scientia Horticulturae*, **31**, 161-174.

BRUHN, D., LEVERENZ, J. W. and SAXE, H. (2000). Effects of tree size and temperature on relative growth rate and its components of *Fagus sylvatica* seedlings exposed to two partial pressures of atmospheric [CO_2]. *New Phytologist*, **146**, 415-425.

BULDER, H. A. M., VAN HASSELT, P. R. and KUIPER, P. J. C. (1987). The effect of temperature on early growth cucumber genotypes differing in genetic adaptation to low-energy conditions. *Scientia Horticulturae*, **31**, 53-60.

BYRNE, M., MURRELL, J. C., OWEN, J. V., KRIEDEMANN, P., WILLIAMS, E. R. and MARAN, G. F. (1997). Identifications and mode of action of quantitative trait loci affecting seedling height and leaf area in *Eucalyptus nitens*. *Theoretical and Applied Genetics*, **94**, 674-681.

BYRNE, M., TIMMERMANS, M., KIDNER, C. and MARTIENSSEN, R. (2001). Development of leaf shape. *Current Opinion in Plant Biology*, **4**, 38-43.

CAMPEN, J. B. and BOT, G. P. A. (2001). Design of a low-energy dehumidifying system for greenhouses. *Journal of Agricultural Engineering Research*, **78**, 65-73.

CARANTA, C., LEFEBVRE, V. and PALLOIX, A. (1997). Polygenic Resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. *Molecular Plant Microbe Interactactions*, **10**, 872-878.

CARANTA, C., PFLIEGER, S., LEFEBVRE, V., DAUBÈZE, A., THABUIS, A. and PALLOIX, A. (2002). TLs involved in the restriction of cucumber mosaic virus (CMV) long-distance movement in pepper. *Theoretical and Applied Genetics*, **104**, 586-591.

CAVACO, A. M., DA SILVI, A. B. and ARRABAÇA, M. C. (2003). Effect of long-term chilling on growth and photosynthesis of the C_4 gramineae *Paspalum dilatatum*. *Physiologia Plantarum*, **119**, 87-96.

CBS. (2006). http://www.cbs.nl/

CLAPHAN, W. M. and MARSH, H. V. (1987). Relationship of vegetative growth and pepper yield. *Canadian Journal of Plant Sciences*, **67**, 521-530.

COLEMAN, J. S., MCCONNAUGHAY, K. D. M. and ACKERLY, D. D. (1994). Interpreting phenotypic variation in plants. *Tree*, **9**, 187-191.

COURTOIS, B., MCLAREN, G., SINHA, P. K., PRASAD, K., YADAV, R. and SHEN, L. (2000). Mapping QTLs associated with drought avoidance in upland rice. *Molecular Breeding*, **6**, 55-66.

CULLEY, T. M., DUNBAR-WALLIS, A. K., SAKAI, A. K., WELLER, S. G., MISHIO, M., CAMPBELL, D. R. and HERZENACH, M. (2005). Genetic variation of ecophysiological traits in two gynodioecious species of *Schiedea* (Caryophyllaceae). *New Phytologist*, **169**, 589-601.

DAY, M. E., GREENWOOD, M. S. and WHITE, A. S. (2001). Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age. *Tree Physiology*, **21**, 1195-1204.

DE SWART, E. A. M., GROENWOLD, R., KANNE, H. J., STAM, P., MARCELIS, L. F. M. and VOORRIPS, R. E. (2004a). Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. *Journal of Horticultural Science & Biotechnology*, **79**, 764-770.

DE SWART, E. A. M., MARCELIS, L. F. M. and VOORRIPS, R. E. (2004b). Search for low temperature tolerant *Capsicum* accessions to conserve energy in greenhouse pepper production. Proceedings of the XIIth Meeting on genetics and Breeding of *Capsicum* and Eggplant. (Voorrips, R. E., Ed.).101-107.

DE SWART, E. A. M., MARCELIS, L. F. M. and VOORRIPS, R. E. (2006). Variation in relative growth rate and growth traits in wild and cultivated *Capsicum* accessions grown under different temperatures. *Journal of Horticultural Science & Biotechnology*, **81**, 1029-1037.

DE ZWART, H. F. and BOT, G. P. A. (1997). Energy saving prospectives of combined heat and power in horticulture in the Netherlands: a simulation study. *Netherlands Journal of Agricultural Science*, **45**, 97-107.

DELI, J. and TIESSEN, H. (1969). Interaction of temperature and light intensity on flowering of *Capsicum frutescens* var. *grossum* sv. 'California Wonder'. *Journal of the American Society for Horticultural Science*, **94**, 349-351.

DEN NIJS, A. P. M. and SMEETS, L. (1987). Analysis of differences in growth of cucumber genotypes under low light conditions in relation to night temperature. *Euphytica*, **36**, 19-31.

DI BENEDETTO, A. H. and COGLIATTI, D. H. (1990). Effects of light intensity and quality on the obligate shade plant *Aglaonema commutatum*. I. Leaf size and shape. *Journal of Horticultural Science & Biotechnology*, **65**, 689-698.

DIELEMAN, J. A., MEINEN, E., MARCELIS, L. F. M., DE ZWART, H. F. and VAN HENTEN, E. J. (2005). Optimisation of CO_2 and temperature in terms of crop growth and energy use. *Acta Horticulturae*, **691**, 149-154.

DJIAN-CAPORALINO, C., PIJAROWSKI, L. , FAZARI, A., SAMSON, M., GAVEAU, L., LEFEBVRE, V., CARANTA, C., PALLOIX, A. and ABAD, P. (2001). High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci Me-3 and Me-4 conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.). *Theoretical and Applied Genetics*, **103**, 592-600.

DODD, I. C., HE, J., TURNBULL, C. G. N., LEE, S. K. and CRITCHLEY, C. (2000). The influence of supra-optimal root-zone temperatures of growth and stomatal conductance in *Capsicum annuum* L. *Journal of Experimental Botany*, **51**, 239-248.

ELBERSE, I. A. M., VANHALA, T. K., TURIN, J. H. B., STAM, P., VAN DAMME, J. M. M. and TIENDEREN, P. H. (2004). Quantitative trait loci affecting growth-related traits in wild barley (*Hordeum spontaneum*) grown under different levels of nutrient supply. *Heredity*, **93**, 22-33.

ELINGS, A., KEMPKES, F. L. K., KAARSEMAKER, R. C., RUIJS, M. N. A., VAN DE BRAAK, N. J. and DUECK, T. A. (2005). The energy balance and energy-saving measures in greenhouse tomato cultivation. *Acta Horticulturae*, **691**, 67-74.

EL-LITHY, M. E., CLERKX, E. J. M., RUYS, G. J., KOORNNEEF, M. and VREUGDENHIL, D. (2004). Quantitative Trait Locus Analysis of Growth-Related Traits in a New Arabidopsis Recombinant Inbred Population. *Plant Physiology*, **135**, 444-458.

ERICKSON, A. N. and MARKHART, A. H. (2001). Flower production, fruit set, and physiology of bell pepper during elevated temperature and vapour pressure deficit. *Journal of the American Society for Horticultural Science*, **126**, 697-702.

ESAU, K. (1965). The leaf. In: *Plant Anatomy*. (Esau, K., Eds). John Wiley and sons, New York, London, Sidney. 467-480.

ESHBAUGH, W. H. (1980). The taxonomy of the genus *Capsicum* (Solanaceae). *Phytologia*, **47**, 153-166.

ESHBAUGH, W. H. (1993). Peppers: history and exploitation of a serendipitous new crop discovery. In: *New Crops*. (Janick, J. and Simon, J. E., Eds). Wiley, New York, USA. 132-139.

EVANS, G. C. (1972). *The Quantitative Analysis of Plant Growth*. Blackwell Scientific Publications, UK, London. 734 pp.

FERRIS, R., SABATTI, M., MIGLIETTA, F., MILLS, R. and TAYLOR, G. (2001). Leaf area is stimulated in Populus by free air CO_2 enrichment (POPFACE), through increased cell expansion and production. *Plant, Cell and Environment*, **24**, 305-315.

FOOLAD, M. R. and LIN, G. Y. (2001). Genetic analysis of cold tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. *Euphytica*, **122**, 105-111.

FRIEND, D. J. C. and HELSON, V. A. (1976). Thermoperiodic effects on the growth and photosynthesis of wheat and other crop plants. *Botanical Gazette*, **137**, 75-84.

GARNIER, E. (1991). Resource capture, biomass allocation and growth in herbaceous plants. *Tree*, **6**, 126-131.

GARNIER, E. (1992). Growth analysis of congeneric annual and perennial grass species. *Journal of Ecology*, **88**, 665-675.

GARNIER, E. and LAURENT, G. (1994). Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist*, **128**, 725-736.

GOUDRIAAN, J. and VAN LAAR, H. H. (1994). *Modelling potential crop growth processes*. Kluwer Academic Publishers, Dordrecht. pp.

GRIME, J. P. and HUNT, R. (1975). Relative growth rate: its range and adaptive significance in a local flora. *Journal of Ecology*, **63**, 393-422.

GRUDA, N. (2005). Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. *Critical Reviews in Plant Sciences*, **24**, 227-247.

GUNN, S. and FARRAR, J. F. (1999). Effects of a 4 °C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. *Functional Ecology*, **13**, 12-20.

HANLEY, M. E., FENNER, M., WHIBLEY, H. and DARVILL, B. (2004). Early plant growth: identifying the end point of the seedling phase. *New Phytologist*, **163**, 61-66.

HEGDE, D. M. (1987). Growth analysis of bell pepper (*Capsicum annuum* L.) in relation to soil moisture and nitrogen fertilization. *Scientia Horticulturae*, **33**, 3-4.

HEISER, C. B. (1964). Los chiles ajies (*Capsicum*) de coste rica y ecuador. *Ciencia*, **7**, 50-57.

HEISER, C. B. and SMITH, P. G. (1953). The cultivated capsicum peppers. *Economic Botany*, **7**, 214-217.

HEKNEBY, M., ANTONÍN, M. C. and SÁNCHEZ-DÍAZ, M. (2006). Frost resistance and biochemical changes during cold acclimation in different annual legumes. *Environmental and Experimental Botany*, **55**, 305-314.

HEUVELINK, E. (1989). Influence of day and night temperature on the growth of young tomato plants. *Scientia Horticulturae*, **38**, 11-22.

HEUVELINK, E. and MARCELIS, L. F. M. (1996). Influence of assimilate supply on leaf formation in sweet pepper and tomato. *Journal of Horticultural Science & Biotechnology*, **71**, 405-414.

HEYWOOD, V. H. (1978). *Flowering plants of the world*. B T Battsford Ltd, London, UK. 335 pp.

HITTALMANI, S., HUANG, N., COURTOIS, B., VENUPRASAD, R., SHASHIDHAR, H. E., ZHUANG, J.-Y., ZHENG, K. L., LIU, G.-F., WANG, G.-C., SIDHU, J. S., SRIVANTANEEYAKUL, S., SINGH, V. P., BAGALI, P. G., PRASANNA, H. C., MCLAREN, G. and KHUSH, G. S. (2003). Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. *Theoretical and Applied Genetics*, **107**, 679-690.

HITTALMANI, S., SHASHIDHAR, H. E., BAGALI, P. G., HUANG, N., SIDHU, J. S., SINGH, V. P. and KHUSH, G. S. (2002). Molecular mapping of quantitative trait loci for plant growth, yield and yield related traits across three diverse locations in a double haploid rice population. *Euphytica*, **125**, 207-214.

HOVENDEN, M. J. (2001). The influence of temperature and genotype on the growth and stomatal morphology of southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *Australian journal of Botany*, **49**, 427-434.

HUH, J. H., KANG, B. C., NAHM, S. H., KIM, S., HA, K. S., H., L. M. and KIM, B. D. (2001). A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.). *Theoretical and Applied Genetics*, **102**, 524-530.

HUND, A., FRASCAROLI, E., LEIPNER, J., JOMPUK, C., STAMP, P. and FRACHEBOUD, Y. (2005). Cold tolerance of the photosynthetic apparatus: pleiotropic relationship between photosynthetic performance and specific leaf area of maize seedlings. *Molecular Breeding*, **16**, 321-331.

HUNT, R. (1982). The Functional Approach to Plant Growth Analysis. In: *Plant Growth curves.* (Hunt, R., Eds). Edward Arnold, London, UK. 5-60.

HUNT, R., CAUSTON, D. R., SHIPLEY, B. and ASKEY, A. P. (2002). A modern tool for classical plant growth analysis. *Annals of Botany*, **90**, 485-488.

HUNT, W. F. and HALLIGAN, G. (1981). Growth and developmental responses of perennial ryegrass grown at constant temperature. I. Influence of light and temperature on growth and net assimilation. *Australian journal of Plant Physiology*, **8**, 181-190.

HURD, R. G. and GRAVES, C. J. (1985). Some effects of air and root temperatures on the yield and quality of glasshouse tomatoes. *Journal of Horticultural Science & Biotechnology*, **60**, 359-371.

JACKSON, P., ROBERTSON, M., COOPER, M. and HAMMER, G. L. (1996). The role of physiological understanding in plant breeding; from a breeding perspective. *Field Crops Research*, **49**, 11-37.

JANSEN, R. C. and STAM, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, **136**, 1447-1455.

JAUHAR, P. P. (2006). Modern biotechnology as an integral supplement to conventional plant breeding: The prospects and challenges. *Crop Science*, **46**, 1841–1859.

JONES, M. P., DINGKUHN, M., ALUKO, G. K. and SEMON, M. (1997). Interspecific *Oryza sativa* L. x *O. Glaberrima* Steud. progenies in upland rice improvement. *Euphytica*, **92**, 237-246.

JOVICICH, E., CANTLIFFE, D. J. and STOFFELLA, P. J. (2004). Fruit yield and quality of greenhouse-grown bell pepper as influenced by density, container, and trellis system. *HortTechnology*, **14**, 507-513.

KAHN, B. A. and LESKOVAR, D. I. (2006). Cultivar and plant arrangement effects on yield and fruit quality of bell pepper. *HortScience*, **41**, 1565-1570.

KANG, B. C., NAHM, S. H., HUH, J. H., YOO, H. S. and KIM, D. D. (2000). Construction of a molecular linkage map in hot pepper. *Acta Horticulturae*, **521**, 165-171.

KANG, B. C., NAHM, S. H., HUH, J. H., YOO, H. S., YU, J. W., LEE, M. H. and KIM, B. D. (2001). An interspecific (*Capsicum annuum* x *C. chinense*) F-2 linkage map in pepper using RFLP and AFLP markers. *Theoretical and Applied Genetics*, **102**, 531-539.

KARIMI, M. M. and SIDDEQUE, K. H. M. (1991). Crop growth and relative growth rates of old and modern wheat cultivars. *Australian Journal of Agricultural Research*, **42**, 13-20.

KLÄRING, H. P., HEIßNER, A. and FINK, M. (1996). Growth of a sweet pepper crop - measurements for modelling. *Acta Horticulturae*, **417**, 107-112.

KOORNNEEF, M. and STAM, P. (2001). Changing paradigms in plant breeding. *Plant Physiology*, **125**, 156-159.

KÖRNER, O., BAKKER, M. J. and HEUVELINK, E. (2004). Daily temperature integration: a simulation study to quantify energy consumption. *Biosystems Engineering*, **87**, 333-343.

KÖRNER, O. and CHALLA, H. (2003). Design for an improved temperature integration concept in greenhouse cultivation. *Computers and electronics in agriculture*, **39**, 39-59.

LAMBERS, H. and POORTER, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research*, **23**, 187-261.

LEE, J. M., NAHM, S. H., KIM, Y. M. and KIM, B. D. (2004). Characterization and molecular genetic mapping of microsatelitte loci in pepper. *Theoretical and Applied Genetics*, **108**, 619-627.

LEFEBVRE, V., DAUBÈZE, A. M., ROUPPE VAN DE VOORT, J., PELEMAN, J., BARDIN, M. and PALLOIX, A. (2003). QTLs for resistance to powdery mildew in pepper under natural and artificial infections. *Theoretical and Applied Genetics*, **107**, 661-666.

LEFEBVRE, V., KUNTZ, M., CAMARA, B. and PALLOIX, A. (1998). The capsanthin-capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Molecular Biology*, **36**, 785-789.

LEFEBVRE, V., PFLIEGER, S., THABUIS, A., CARANTA, C., BLATTES, A., CHAUVET, J. C., DAUBÈZE, A. M. and PALLOIX, A. (2002). Towards the saturation of the pepper linkage mao by alignment of three intraspecific maps including known-function genes. *Genome*, **45**,

LIVINGSTONE, K. D., LACKNEY, V. K., BLAUTH, J. R. and VAN WIJK, R. (1999). Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. *Genetics*, **152**, 1183-1202.

LOVEYS, B. R., SCHEURWATER, I., PONS, T. L., FITTER, A. H. and ATKIN, O. K. (2002). Growth temperature influences the underlying components of relative growth rate: an investigation using inherent fast- and slow-growing plant species. *Plant, Cell and Environment*, **25**, 975-987.

LUND, J. B., ANDREASSEN, A., OTTOSEN, C. O. and AASLYNG, J. M. (2006). Effect of a dynamic climate on energy consumption and production of *Hibiscus rose-sinensis* L. in greenhouses. *HortScience*, **41**, 384-388.

MALOOF, J. (2003). QTL for plant growth and morphology. *Current Opinion in Plant Biology*, **6**, 85-90.

MANSUR, L. M., ORF, J. H., CHASE, K., JARVIK, T., CREGAN, P. B. and LARK, K. G. (1996). Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Science*, **36**, 1327-1336.

MARCELIS, L. F. M., HEUVELINK, E., BAAN HOFMAN-EIJER, L. R., DEN BAKKER, J. and XUE, L. B. (2004). Flower and fruit abortion in sweet pepper in relation to source and sink strength. *Journal of Experimental Botany*, **55**, 2261-2268.

MARSHALL, J. K. (1968). Methods for leaf area measurement of large and small samples. *Photosynthetica*, **2**, 41-47.

MEERTS, P. and GARNIER, E. (1996). Variation in relative growth rate and its components in the annual *Polygonum aviculare* in relation to habitat disturbance and seed size. *Oecologia*, **108**, 438-445.

MERCADO, J. A., REID, M. S., VALPUESTA, V. and QUESADA, M. A. (1997). Metabolic changes and susceptibility to chilling stress in *Capsicum annuum* plants grown at suboptimal temperature. *Australian journal of Plant Physiology*, **24**, 759-767.

MESHRAM, L. D. and MUKEWAR, L. D. (1986). Heterosis studies in chilli (*Capsicum annuum* L.). *Scientia Horticulturae*, **28**, 219-225.

MITCHELL, K. J. (1956). Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *New Zealand Journal of Science and Technology*, **38**, 203-216.

MOLCHOVA, E. and MICHAILOVA, M. (1982). On the interspecific crossability between *Capsicum annuum* L. and *Capsicum pendulum* Willd. (sin baccatum). *Capsicum Newsletter*, **1**, 38-40.

MONTERO, F. J., DE JUAN, J. A., CUESTA, A. and BRASA, A. (2000). Nondestructive methods to estimate leaf area in *Vitis vinifera* L. *HortScience*, **35**, 696-698.

NESMITH, D. S. (1992). Estimating summer squash leaf area nondestructively. *HortScience*, **27**, 77.

NEVADO, M. E. and CROSS, H. Z. (1990). Diallel analysis of relative growth-rates in maize synthetics. *Crop Science*, **30**, 549-552.

NIEUWHOF, M., JANSEN, J. and VAN OEVEREN, J. C. (1993). Genotypic variation for relative growth rate and other growth parameters in tomato (*Lycopersicun esculentum* Mill.) under low energy conditions. *Journal of Genetics and Breeding*, **47**, 35-44.

NILWIK, H. J. M. (1980a). Photosynthesis of whole sweet pepper plants 1. Response to irradiance and temperature as influenced by cultivation conditions. *Photosynthetica*, **14**, 373-381.

NILWIK, H. J. M. (1980b). Photosynthesis of whole sweet pepper plants 2. Response to CO_2 carbon dioxide concentration, irradiance and temperature as influenced by cultivation conditions. *Photosynthetica*, **14**, 382-391.

NILWIK, H. J. M. (1981a). Growth analysis of sweet pepper (*Capsicum annuum* L.). 1. The influence of irradiance and temperature under glasshouse conditions in winter. *Annals of Botany*, **48**, 129-136.

NILWIK, H. J. M. (1981b). Growth analysis of sweet pepper (*Capsicum annuum* L.). 2. Interacting effects of irradiance, temperature and plant age in controlled conditions. *Annals of Botany*, **48**, 137-145.

OLEKSYN, J., MODRZYNSKI, J., TJOELKER, M. G., ZYTKOWIAK, R., REICH, P. B. and KAROLEWSKI, P. (1998). Growth and physiology of *Picea abies* populations from elevated transects: common garden evidence for altitude ecotypes and cold adaptation. *Functional Ecology*, **12**, 573-590.

ONUS, A. N. and PICKERSGILL, B. (2004). Unilateral incompatibility in *Capsicum* (Solanaceae): occurrence and taxonomic distribution. *Annals of Botany*, **94**, 289-295.

ORTEGA, R. G., GUTIERREZ, M. and CAVERO, J. (2004). Plant density influences marketable yield of directly seeded 'Piquillo' pimiento pepper. *HortScience*, **39**, 1584-1587.

OTTOSEN, C. O., ROSENQVIST, E. and SØRENSEN, L. (2001). Energiebesparing door intelligente klimaatbeheersing. *Groenten & Fruit*, **week 50**, 32-33.

OTTOSEN, C. O., ROSENQVIST, E. and SØRENSEN, L. (2003). Effect of a dynamic climate control on energy saving, yield and shelf life of spring production of bell peppers (*Capsicum annuum* L.). *European Journal of Horticultural Science*, **68**, 26-31.

OWONA, S. M. (2005). *Introgressions of Lycopersicon pennellii improve growth and development of greenhouse tomatoes*. Thesis Wageningen University, 126 pp.

PANTA, G. R. and NESMITH, D. S. (1995). A model for estimating area of muskmelon leaves. *HortScience*, **30**, 624-625.

PARAN, I., AFTERGOOT, E. and SHIFRISS, C. (1998). Variation in *Capsicum annuum* revealed RAPD and AFLP markers. *Euphytica*, **99**, 167-173.

PARAN, I., I., G. and ZAMIR, D. (1997). QTL analysis of morphological traits in an tomato recombinant inbred line population. *Genome*, **40**, 242-248.

PARAN, I., ROUPPE VAN DE VOORT, J., LEFEBVRE, V., JAHN, M., LANDRY, L., VAN SCHRIEK, M., TANYOLAC, B., CARANTA, C., BEN CHAIM, A., LIVINGSTONE, K. D., PALLOIX, A. and PELEMAN, J. (2004). An integrated genetic linkage map of pepper (*Capsicum* spp.). *Molecular Breeding*, **13**, 251-261.

PATTERSON, D. T. (1993). Effect of day and night temperature on goatsrue (*Galega officinalis*) and alfalfa (*Medicago savita*) growth. *Weed Science*, **41**, 38-45.

PAUL, N. K. (1992). Variation in morpho-physiological characters in forage rape (*Brassica napus* L.). 1. Growth attributes and leaf characteristics. *Genetica Polonica*, **33**, 35-43.

PAYNE, R., MURRAY, D., HARDING, S., BAIRD, B., SOUTAR, D. and LANE, P. (2002). *GenStat® for Windows(tm) 6th Edition Introduction. VSN International. ISBN 1-904375-06-5*. pp.

PÉREZ-GRAJALES, M., GONZÁLEZ-HERNÁNDEZ, V. A., MENDOZA-CASTILLO, M. C. and PEÑA-VALDIVIA, C. (2004). Physiological characterization of Manzano Hot Pepper (*Capsicum pubescens* R & P) landraces. *Journal of the American Society for Horticultural Science*, **129**, 88-92.

PERSAUD, N., GANDAH, M., OUATTARA, M. and MOKETE, N. (1993). Estimating leaf area of Pearl Millet from linear measurements. *Agronomy Journal*, **85**, 10-12.

PET, G. (1983). Minder energie voor paprika? *Groenten & Fruit*, **1 april**, 75-77.

PET, G. (1985). Verband tussen kwaliteit stuifmeel en vroegheid bij paprika. *Groenten & Fruit*, **25 oktober**, 42-43.

PHILLIPS, R. L. (2006). Genetic tools from nature and the nature of genetic tools. *Crop Science*, **46**, 2245-2252.

PICKERSGILL, B. (1971). Relationship between weedy and cultivated forms in some species of chili peppers (Genus *Capsicum*). *Evolution*, **25**, 683-691.

PICKERSGILL, B. (1988). The genus *Capsicum*: a multidisciplinary approach to the taxonomy of cultivated and wild plants. *Biologisches Zenttralblatt*, **107**, 381-389.

PICKERSGILL, B. (1997). Genetic resources and breeding of *Capsicum* spp. *Euphytica*, **96**, 129-133.

POETHIG, R. S. (1997). Leaf morphogenesis in flowering plants. *The Plant Cell*, **9**, 1077-1087.

POORTER, H. (1989a). Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: *Causes and Consequences of Variation in Growth Rate and Productivity in Higher Plants*. (Lambers, H., Cambridge, M. L., Koning, H. and Pons, T. C., Eds). SPB Academic Publishing b.v., The Hague, The Netherlands. 45-68.

POORTER, H. (1989b). Plant growth analysis: Towards a synthesis of the classical and the functional approach. *Physiologia Plantarum*, **75**, 237-244.

POORTER, H. and REMKES, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*, **83**, 553-559.

POORTER, H. and VAN DER WERF, A. (1998). Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? In: *Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences*. (Lambers, H., Poorter, H. and Van Vuuren, M. M. I., Eds). Backhuys Publishers, Leiden, The Netherlands. 309-336.

POORTER, H., VAN RIJN, C. P. E., VANHALA, T. K., VERHOEVEN, K. J. F., DE JONG, Y. E. M., STAM, P. and LAMBERS, H. (2005). A genetic analysis of relative growth rate and underlying components in *Hordeum spontaneum*. *Oecologia*, **142**, 360–377.

POPOVSKY, S. and PARAN, I. (2000). Molecular genetics of the y locus in pepper: its relation to capsanthin-capsorubin synthase and to fruit color. *Theoretical and Applied Genetics*, **101**, 86-89.

POTTER, J. R. and JONES, J. W. (1977). Leaf area partitioning as an important factor in growth. *Plant Physiology*, **59**, 10-14.

POULOS, J. M. (1993). *Capsicum* L. In: *Plant Resources of South-East Asia 8: Vegetables*. (Siemonsa, J. S. and Piluek, K., Eds). Prosea, Bogor, Indonesia. 136-140.

PRESSMAN, E., MOSHKOVITCH, H., ROSENFELD, K., SHAKED, R., GAMLIEL, B. and ALONI, B. (1998). Influence of low night temperatures on sweet pepper flower quality and the effect of repeated pollinations, with viable pollen, on fruit setting. *Journal of Horticultural Science & Biotechnology*, **73**, 131-136.

PRESSMAN, E., SHAKED, R. and FIRON, N. (2006). Exposing pepper plants to high day temperatures prevents the adverse low night temperature symptoms. *Physiologia Plantarum*, **126**, 618-626.

PRICE, A. H. (2006). Believe it or not, QLTs are accurate! *Trends in Plant Science*, **11**, 213-216.

PRITSA, T. S., VOYIATZIS, D. G., VOYIATZI, C. J. and SOTIRIOU, M. S. (2003). Evaluation of vegetative growth traits and their relation to time to first flowering of olive seedlings. *Australian Journal of Agricultural Research*, **54**, 371-376.

QUIRIN, E. A., OGUNDIWIN, E. A., PRINCE, J. P., MAZOUREK, M., BRIGGS, M. O., CHLANDA, T. S., KIM, K.-T., FALISE, M., KANG, B.-C. and JAHN, M. M. (2005). Development of sequence characterized amplified region (SCAR) primers for the detection of *Phyto.5.2*, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. *Theoretical and Applied Genetics*, **110**, 605-612.

RANDLE, W. M. and HONMA, S. (1980). Inheritance of low temperature emergency in *Capsicum baccatum* var. *pendulum*. *Euphytica*, **29**, 331-335.

RAO, G. U., BEN CHAIM, A., BOROVSKY, Y. and PARAN, I. (2003). Mapping of yield-related QTLs in pepper in an interspecific cross of *Capsicum annuum* and *C. fructescens*. *Theoretical and Applied Genetics*, **106**, 1457-1466.

RAY, J. C. and SINGH, R. P. (1989). Leaf area estimation in *Capsicum* (*Capsicum annuum* L.). *Scientia Horticulturae*, **39**, 181-188.

REISER, L., MUELLER, L. A. and RHEE, S. Y. (2002). Surviving in a sea of data: a survey of plant genome data resources issues in building data management systems. *Plant Molecular Biology*, **48**, 59-74.

REMINGTON, D. L. and PURUGGANAN, M. C. (2003). Candidate gens, quantitaitve trait loci, and functional trait evolution in plants. *International Journal of Plant Sciences*, **164**, s7-s20.

RYLSKI, I. (1972). Effect of early environment on flowering in pepper (*Capsicum annuum* L.). *Journal of the American Society for Horticultural Science*, **97**, 648-651.

SALVI, S. and TUBEROSA, R. (2005). To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Science*, **10**, 297-304.

SCHWARZ, D. and KLÄRING, H. P. (2001). Allometry to estimate leaf area of tomato. *Journal of Plant Nutrition*, **24**, 1297-1309.

SERQUEN, F. C. and STAUB, J. E. (1997). Mapping and QTL analysis of horticultural traits in an narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. *Molecular Breeding*, **3**, 257-268.

SEZEN, S. M., YAZAR, A. and EKER, S. (2006). Effect of drip irrigation regimes on yield and quality of field grown bell pepper. *Agricultural Water Management*, **81**, 115-131.

SHIPLEY, B. (2006). Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. *Functional Ecology*, **20**, 565-574.

SHIVASHANKAR, S., IYER, R. and VIJAYAKUMAR, K. (1986). A non-destructive method of estimating leaf area in pepper seedlings. *Indian Journal of Agricultural Sciences*, **56**, 619-621.

SI, P. and THURLING, N. (2001a). A greater relative growth rate of *Brassica rapa* L. at low temperatures increases biomass at anthesis. *Australian Journal of Agricultural Research*, **52**, 645-652.

SI, P. and THURLING, N. (2001b). Genetic improvement of pre-anthesis growth of turnip rape (*Brassica rapa* L.) at low temperature. *Australian Journal of Agricultural Research*, **52**, 653–660.

SI, Y. and HEINS, R. D. (1996). Influence of day and night temperatures on sweet pepper seedling development. *Journal of the American Society for Horticultural Science*, **121**, 699-704.

SIGRIMIS, N., ANASTASIOU, A. and RERRAS, N. (2000). Energy saving in greenhouses using temperature integration: a simulation survey. *Computers and electronics in agriculture*, **26**, 321-341.

SIMON, J. E., CHADWICK , A. F. and CRAKER, L. E. (1984). *Herbs: an Indexed Bibliography, 1971 - 1980: the Scientific Literature on Selected Herbs and Aromatic and Medicinal Plants of the Temperate Zone*. Elsevier Publishers, Amsterdam, The Netherlands. 770 pp.

SLAFER, G. A. (2003). Genetic basis of yield as viewed from a crop physiologist's perspective. *Annuals of Applied Biology*, **142**, 117-128.

SMEETS, L. and GARRETSEN, F. (1986). Inheritance of growth characteristics of tomato (*Lycopericum esculentum* Mill.) under low energy conditions. *Euphytica*, **35**, 877-887.

SMITH, P. G. and HEISER, C. B. (1957). Taxonomy of *Capsicum sinense* Jacq. and the geographical distribution of the cultivated *Capsicum* species. *Bulletin of the Torrey Botanical Club*, **84**, 413-420.

SMITH, P. G., VILLALON, B. and VILLA, P. L. (1987). Horticultural classification of peppers grown in the United States. *HortScience*, **22**, 11-13.

SOKAL, R. R. and ROHLF, F. J. (1981). *Biometry*. W.H. Freeman and Company, New York. pp.

SOLDATI, A., STEHLI, A. and STAMP, P. (1999). Temperature adaptation of tropical highland maize (*Zea mays* L.) during early growth and in controlled conditions. *European Journal of Agronomy*, **10**, 111-117.

SPARNAAIJ, L. D., KOEHORST-VAN PUTTEN, H. J. J. and BOS, I. (1996). Component analysis of plant dry matter production: a basis for selection of breeding parents as illustrated in carnation. *Euphytica*, **90**, 183-194.

SPITTERS, C. J. T. and KRAMER, T. (1986). Differences between spring wheat cultivars in early growth. **35**, 273-292.

STEHLI, A., SOLDATI, A. and STAMP, P. (1999). Vegetative performance of tropical highland maize (*Zea mays* L.) in the field. *Journal of Agronomy & Crop Science*, **183**, 193-198.

STEWART, D. W. and DWYER, L. M. (1999). Mathematical characterisation of leaf shape and area in maize hybrids. *Crop Science*, **39**, 422-427.

STIRLING, C. M., HEDDELL-COWIE, M. and JONES, M. L. (1998). Effect of elevated CO_2 and temperature on growth and allometry of five native fast-growing annual species. *New Phytologist*, **140**, 343-354.

SUGIYAMA, N. and OOZONO, M. (1999). Leaf initiation and development in criphead and butterhead lettuce plants. *Journal of the Japanese Society of Horticultural Science*, **68**, 1118-1123.

SWINKELS, G. L. A. M., SONNEVELD, P. J. and BOT, G. P. A. (2001). Improvement of greenhouse insulation with restricted transmission loss through zagzag covering material. *Journal of Agricultural Engineering Research*, **79**, 91-97.

TAYLOR, G., CEULEMANS, R., FERRIS, R., GARDNER, S. D. L. and SHAO, B. Y. (2001). Increased leaf area expansion of hybrid poplar in elevated CO_2. From controlled environments to open-top chambers and to FACE. *Environmental Pollution*, **115**, 463-472.

TAYLOR, G., TRICKER, P. J., ZHANG, F. Z., ALSTON, V. J., MIGLIETTA, F. and KUZMINSKY, E. (2003). Spatial and temporal effects of free-air CO_2 enrichment (POPFACE) on leaf growth, cell expansion, and cell production in a closed canopy of poplar. *Plant Physiology*, **131**, 177-185.

TER STEEGE, M. W., DEN OUDEN, F. M., LAMBERS, H., STAM, P. and PEETERS, A. J. M. (2005). Genetic and physiological architecture of early vigor in *Aegilops tauschii,* the D-genome donor of hexaploid wheat. A Quantitative Trait Loci analysis. *Plant Physiology*, **139**, 1078–1094.

THABUIS, A., PALLOIX, A., SERVIN, B., DAUBÈZE, A. M., SIGNORET, P., HOSPITAL, F. and LEFEBVRE, V. (2004). Marker-assisted introgression of 4 *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. *Molecular Breeding*, **14**, 9-20.

THOMAS, S. C. and BAZZAZ, F. A. (1996). Elevated CO_2 and leaf shape: are dandelions getting toothier? *American Journal of Botany*, **83**, 106-111.

THORVALDSSON, G. and MARTIN, R. C. (2003). Growth response of seven perennial grass species to three temperature regimes applied at two growth stages. *Acta Agriculturæ Scandinavica Section B, Soil and Plant Science*, **54**, 14-22.

TJOELKER, M. G., OLEKSYN, J. and BREICH, P. B. (1998). Temperature and ontogeny mediate growth response to elevated CO_2 in seedlings of five boreal tree species. *New Phytologist*, **140**, 197-210.

VAN ARENDONK, J. J. C. M. and POORTER, H. (1994). The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant, Cell and Environment*, **17**, 963-970.

VAN DER KNAAP, E. and TANKSLEY, S. D. (2001). Identification and characterisation of a novel locus controlling early fruit development in tomato. *Theoretical and Applied Genetics*, **103**, 353-358.

VAN DER KNIJFF, A. and BENNINGA, J. (2003). *Energie in de glastuinbouw van Nederland, Ontwikkelingen in de sector en op de bedrijven t/m 2002*. Landbouw-Economisch Instituut LEI, Wageningen-UR, Den Haag, the Netherlands. 65 pp.

VAN DER KNIJFF, A., BENNINGA, J., REIJNDERS, C. and NIENHUIS, J. (2006). *Energie in de glastuinbouw van Nederland Ontwikkelingen in de sector en op de bedrijven tot en met 2004*. Landbouw-Economisch Instituut LEI, Wageningen-UR, Den Haag, the Netherlands. 77 pp.

VAN DER PLOEG, A. and HEUVELINK, E. (2005). Influence of sub-optimal temperature on tomato growth and yield: a review. *Journal of Horticultural Science & Biotechnology*, **80**, 652-659.

VAN DER PLOEG, A. and HEUVELINK, E. (2006). The influence of temperature on growth and development of chrysanthemum cultivars: a review. *Journal of Horticultural Science & Biotechnology*, **81**, 174-182.

VAN DER PLOEG, A., KULARATHNE, R. J. K. N., CARVALHO, S. M. P. and HEUVELINK, E. (2007). Variation Between Cut Chrysanthemum Cultivars in Response to Suboptimal Temperature. *Journal of the American Society for Horticultural Science*, **132,** 52-59.

VAN DER PLOEG, A., SMID, H. G. and HEUVELINK, E. (2005). Cultivar differences in temperature demand of cut chrysanthemum. *Acta Horticulturae*, **691**, 91-98.

VAN IERSEL, M. W. and LINDSTROM, O. M. (1999). Temperature response of whole-plant CO2 exchange rates of three rates of magnolia cultivars. *Journal of the American Society for Horticultural Science*, **124**, 277-282.

VAN OOIJEN, J. W., BOER, M. P., JANSEN, R. C. and MALIEPAARD, C. (2002). MapQTL®4.0, software for the calculation of QTL positions on the genetic maps. Plant Research International, Wageningen, The Netherlands.

VAN OOIJEN, J. W. and VOORRIPS, R. E. (2001). Joinmap®3.0, software for calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands.

VAN RIJN, C. P. E. (2001). *A physiological and genetic analysis of growth characteristics in Hordeum spontaneum*. Thesis: Utrecht University, 125 pp.

VAN RIJN, C. P. E., HEERSCHE, I., VAN BERKEL, Y. E. M., NEVO, E., LAMBERS, H. and POORTER, H. (2000). Growth characteristics in *Hordeum spontaneum* populations from different habitats. *New Phytologist*, **146**, 471-481.

VERHEUL, M. J., PICATTO, C. and STAMP, P. (1996). Growth and development of maize (*Zea mays* L.) seedlings under chilling conditions in the field. *European Journal of Agronomy*, **5**, 31-43.

VERHOEVEN, K. J. F., VANHALA, T. K., BIERE, A., NEVO, E. and VAN DAMME, J. M. M. (2004). The genetic basis of adaptive population differentiation: a quantitative trait locus analysis of fitness traits in two wild barley populations from contrasting habitats. *Evolution*, **58**, 270-283.

VERWIJST, T. and WEN, D. Z. (1996). Leaf allometry of *Salix viminalis* during the first growing season. *Tree Physiology*, **16**, 655-660.

VILLA-CASTORENA, M., ULERY, A. L., CATALÁN-VALENCIA, E. A. and REMMENGA, M. D. (2003). Salinity and nitrogen rate effects on the growth and yield of chile pepper plants. *Soil Science Society of America Journal*, **67**, 1781-1789.

VILLAR, R., VENEKLAAS, E. J., JORDANO, P. and LAMBERS, H. (1998). Relative growth rate and biomass allocation in 20 *Aegilops* (Poaceae) species. *New Phytologist*, **140**, 425-437.

VOORRIPS, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, **93**, 77-78.

VOORRIPS, R. E., FINKERS, R., SANJAYA, L. and GROENWOLD, R. (2004). QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics*, **109**, 1275–1282.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DER LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., M., K. and ZABEAU, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research*, **23**, 4407-4414.

WANG, L. H., ZHANG, B. X., LEFEBVRE, V., HUANG, S. W., DAUBÈZE, A. M. and PALLOIX, A. (2004). QTL analysis of fertility restoration in cytoplasmic male sterile pepper. *Theoretical and Applied Genetics*, **109**, 1058–1063.

WARDLAW, C. W. (1968). Leaves and buds: further observations. In: *Morphogenesis in plants. A contemporary study*. (Wardlaw, C. W., Eds). Methuen & Co TLD, London. 181-225.

WEIH, M., RÖNNBERG-WÄSTLJUNG, A.-C. and GLYNN, C. (2006). Genetic basis of phenotypic correlations among growth traits in hybrid willow (*Salix dasyclados* × *S. viminalis*) grown under two water regimes. *New Phytologist*, **170**, 467–477.

WHAN, B. R., CARLTON, G. P. and ANDERSON, W. K. (1991). Potential for increased early vigour and total biomass in spring wheat. I. Identification of genetic improvement. *Australian Journal of Agricultural Research*, **42**, 347-361.

XIONG, F., MUELLER, E. C. and DAY, T. A. (2000). Photosynthetic and respiratory acclimation and growth response of antarctic vascular plants to contrasting temperature regimes. *American Journal of Botany*, **87**, 700-710.

XU, Y. (1997). Quantitative trait loci: separating, pyramiding, and cloning. *Plant Breeding Reviews*, **15**, 85-139.

YIN, X., KROPFF, M. J. and STAM, P. (1999a). The role of ecophysiological models in QTL analysis: the example of specific leaf area in barley. *Heredity*, **82**, 415-421.

YIN, X., STAM, P., DOURLEIJN, C. J. and KROPFF, M. J. (1999b). AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. *Theoretical and Applied Genetics*, **99**, 244-253.

ZHANG, Z.-H., QU, X.-S., WAN, S., CHEN, L.-H. and ZHU, Y.-G. (2005). Comparison of QTL controlling seedling vigour under different temperature conditions using recombinant inbred lines in rice (*Oryza sativa*). *Annals of Botany*, **95**, 423–429.

ZIJLSTRA, S., PURIMAHUA, C. and LINDHOUT, P. (1991). Pollen tube growth in interspecific crosses between *Capsicum* species. *HortScience*, **26**, 585-586.

ZYGIER, S., BEN CHAIM, A., EFRATI, A., KALUZKY, G., BOROVSKY, Y. and PARAN, I. (2005). QTLs mapping for fruit size and shape in chromosomes 2 and 4 in pepper and a comparison of the pepper QTL map with that of tomato. *Theoretical and Applied Genetics*, **111**, 437–445.

Summary

Sweet pepper (*Capsicum annuum* L.) is an important greenhouse crop in the Netherlands. In 2005, the production area of sweet pepper in the Netherlands exceeded 1230 ha. *Capsicum* plants generally require relatively high temperatures to grow and produce fruit. As a consequence, the energy input needed to grow sweet pepper is high, approximately 42 m^3 natural gas per m^2 of greenhouse area per year. Social and political demands cause the horticultural sector to search for ways to improve the energy efficiency in horticultural production.

Scientific research has contributed in several ways to the increase of energy efficiency in Dutch horticulture: technical measures and dynamic climate control - using the ability of a crop to compensate for temperature changes within a limited period – have been shown to lead to a significant energy reduction compared to standard climate conditions.

Another approach to save energy is to lower the greenhouse temperature. To do so cultivars are needed that tolerate lowered temperatures without loss of yield. In this thesis the possibility to breed sweet pepper cultivars for adaptation to cooler growing conditions is studied. To this end, the physiology of growth and development of pepper and related *Capsicum* species were observed on young plants, and the inheritance of growth related traits was analysed. Selection for rapid growth in the vegetative growth phase of *Capsicum* is most likely an efficient method for the selection of plants with good growth and dry mass production in the generative phase (Chapter 6).

To be able to perform repeated non-destructive measurements of leaf area on single plants without harvesting and sacrificing them (Chapter 5), a method was developed to estimate leaf area from non-destructive measurements in *C. annuum* (Chapter 2). Leaf area could be predicted from the product of leaf length and width ($\alpha \cdot L \cdot W$); by the addition of both linear and quadratic leaf width terms the model ($Area = 0.61906 \cdot L \cdot W + 0.2060 \cdot W^2 - 0.5142 \cdot W$) became independent of plant age and accession and could be used for leaf area estimation during the whole vegetative phase as well as at the start of the generative growth phase of *Capsicum*. It was demonstrated that this model predicts total plant leaf area accurately, even when the length and width of only 25% of the leaves were measured. The value of this method was

shown in Chapter 5 where a large number of genetically unique F_2 and F_3 plants could be measured several times.

In Chapter 3 the influence of lowered temperature on relative growth rate (RGR; increase in dry mass per unit biomass per unit of time) and its underlying physiological and morphological traits are described in a group of wild and cultivated *Capsicum* accessions, using a growth analysis at two temperature regimes (21.1°/18.7°C and 17.3°/14.7°C; day/night). The growth related traits were net assimilation rate (NAR; increase in dry mass per unit leaf area per unit time), the leaf area ratio (LAR; the leaf area per unit of total plant dry mass), specific leaf area (SLA; leaf area per unit leaf dry mass) and leaf mass fraction (LMF; the fraction of total plant dry mass allocated to the leaves). The relative difference in RGR between plants grown at different temperature conditions was used as a measure for tolerance to lowered temperatures.

Based on this difference in RGR, some *Capsicum* accessions were shown to be better adapted to lowered temperatures than others, indicating an opportunity to breed for lowered temperature tolerance in *Capsicum*. Under both temperature regimes NAR proved to be the most important factor affecting the variation in RGR among the *Capsicum* accessions. NAR and LAR were negatively correlated in a group of ten *Capsicum* accessions. Some accessions combined a relatively high NAR and a relatively high LAR under lowered temperatures resulting in a limited effect of temperature on RGR. Thus, as we argued in Chapter 6, the trade-off between NAR and LAR in many accessions does not exclude the possibility that high LAR and high NAR can be combined, resulting in a single genotype with an increased RGR.

Subsequently, we tried to identify which traits can be used to simplify the selection for RGR in breeding programmes (Chapter 4). RGR provides important information about general plant growth and development but is difficult to assess in breeding programs. By identifying physiological and morphological traits that are related with RGR but are easier to obtain than RGR itself, indirect selection for RGR may become feasible in practical breeding. The relationship between RGR and a number of physiological and morphological plant traits (relative leaf growth rate (RLGR), leaf, stem and total fresh and dry mass, plant height, leaf area, number of leaves) was studied in

broader collection of *Capsicum* accessions as well as in a group of sweet pepper cultivars. The relations between RGR and leaf, stem and total fresh and dry mass, plant height, leaf area, number of leaves found in the collection of *Capsicum* accessions was not found in the group of sweet peppers, limiting the practical use of these traits as a measure for RGR. RLGR, on the other hand, was strongly correlated with RGR. Also, RLGR can be calculated from non-destructive leaf area measurements using the model described in Chapter 2. This makes RLGR a valuable, easy-to-measure trait that can be used as an indirect measure for RGR (Chapter 6).

In Chapter 4, we also made a comparison between the variation in the ability to adapt to lowered temperatures in cultivated sweet pepper varieties on the one hand and the variation found in the broader collection of *Capsicum* accessions studied in Chapter 3 on the other hand; within the group of sweet peppers, the variation for lower temperature tolerance was only limited. We concluded that further exploitation of the genetic variation for lowered temperature tolerance in wild relative *Capsicum* species is recommendable in order to breed cultivars with increased energy efficiency.

The possibility to use this physiological and morphological information on *Capsicum* in breeding programmes for lowered temperature tolerance is described in Chapter 5. The inheritance of RLGR and several other physiological and morphological plants traits were examined in an intraspecific *C. annuum* F_2 population. The most important agronomic trait for which a QTL could be identified in the F_2 population and validated in a group of 25 selected F_3 lines was RLGR. In addition, 14 more QTLs related to plant growth and development were identified; the effect of six of them (e.g. leaf mass fraction (LMF), dry mass, number of leaves) could be validated in a set of F_3 lines. In the sweet pepper cultivars studied in Chapter 4 traits of these morphological QTLs were not correlated to RGR and are therefore most likely of limited value with respect to plant growth, although they might be more valuable with respect to plant development. Most important, this study shows that marker assisted selection can be used to breed for growth and development in *Capsicum*.

In short, this thesis presents new knowledge and tools for breeding lowered temperature tolerant sweet pepper cultivars, thus enabling a reduction in energy use and CO_2 emission.

Samenvatting

Paprika (*Capsicum annuum* L.) is een belangrijk glastuinbouwgewas in Nederland. Het totale productieareaal van paprika besloeg in 2005 ruim 1230 ha. *Capsicum*, het genus waar o.a. paprika toe behoort, is een gewas dat hoge temperaturen nodig heeft voor groei en vruchtproductie. Voor de productie van paprika zijn telers in Nederland dan ook aangewezen op verwarmde kassen. Het merendeel van de energie die nodig is voor het verwarmen van de kas wordt geleverd door de verbranding van aardgas. Per jaar is er ongeveer 42 m^3 aardgas per m^2 nodig voor de productie van paprika.

De sector en overheid hebben afspraken gemaakt om de energie efficiëntie te verhogen en de CO_2 uitstoot te beperken. Wetenschappelijk onderzoek heeft op verschillende manieren bijgedragen aan het verhogen van de energie efficiëntie in de glastuinbouw door bijvoorbeeld beweegbare schermen, warmteopslagtanks en gebruikmaking van temperatuurintegratie (het gebruikmaken van de eigenschap van een gewas om zich aan korte perioden van variërende temperaturen aan te passen).

Een andere methode van energiebesparing in de glastuinbouw is het simpelweg verlagen van de dag- en nachttemperaturen. Dit heeft doorgaans echter grote nadelige gevolgen voor de opbrengst en vruchtkwaliteit en kan dus alleen worden uitgevoerd wanneer de gewassen zijn aangepast aan die lagere temperaturen. Tot nu toe zijn de genetische bronnen binnen het genus *Capsicum* hiervoor slechts in beperkte mate aangesproken. Het is niet onwaarschijnlijk dat genetische variatie voor lage temperatuurtolerantie aanwezig is in dit geslacht, daar sommige soorten voorkomen in koele habitats. In dit proefschrift wordt onderzocht of het mogelijk is de lage temperatuurtolerantie van paprika te verhogen middels veredeling. Hiertoe werden zowel de fysiologie van de groei en ontwikkeling als de genetica van hiermee gerelateerde eigenschappen bij *Capsicum* bestudeerd. Het onderzoek is uitgevoerd aan jonge planten daar deze makkelijker en efficiënter meetbaar zijn dan oudere planten. Daarbij is de verwachting dat de groei van jonge planten voldoende informatie geeft over de groei van planten tijdens de productiefase (Hoofdstuk 6).

In Hoofdstuk 2 is een methode ontwikkeld om aan de hand van eenvoudige metingen van bladlengte (L) en bladbreedte (B) van individuele bladeren, het totale bladoppervlak van een plant te berekenen. Deze methode maakt het mogelijk om het totale bladoppervlak van individuele planten meerdere malen te bepalen zonder deze te oogsten. Bladoppervlakte kan (behoudens een constante) in eerste instantie worden berekend als het product van de bladlengte en bladbreedte (α•L•B). Dit model compenseert echter niet voor de vormverandering van individuele bladeren gedurende hun ontwikkeling. Door de uitbreiding van dit model met een lineaire en een extra kwadratische term (B en B^2) blijkt het model onafhankelijk te worden van plant leeftijd en (onder)soort. Dit model ziet er als volgt uit: bladoppervlak = $0.61906 \bullet L \bullet B + 0.2060 \bullet B^2 - 0.5142 \bullet B$. Hiermee kan het totale bladoppervlak van planten zeer nauwkeurig worden bepaald, zelfs wanneer van slechts 25% van de bladeren de lengte en breedte wordt gemeten. In Hoofdstuk 5 van dit proefschrift is deze methode met succes toegepast om meerdere bladoppervlaktemetingen aan genetisch unieke F_2 en F_3 planten doen.

Een belangrijke component van het in dit proefschrift beschreven onderzoek is de studie van de fysiologie van de groei en ontwikkeling van *Capsicum.* Alvorens dit onderzoek te bespreken is het van belang een aantal begrippen te introduceren. De relatieve groeisnelheid (RGR, relative growth rate) is de toename in drooggewicht per gewichtseenheid per dag. RGR kan worden 'ontbonden' in het product van twee andere grootheden. De eerste hiervan is de netto assimilatiesnelheid (NAR, net assimilation rate: toename in plant drooggewicht per eenheid bladoppervlak per dag). Dit is een fysiologische component die het verschil weergeeft tussen de gewichtstoename van de plant door fotosynthese en het gewichtsverlies door ademhaling. De tweede component, een morfologische, is de bladoppervlakte ratio (LAR, leaf area ratio: eenheden bladoppervlak per eenheid plant drooggewicht). De LAR op zijn beurt is het product van de specifieke bladoppervlakte (SLA, specific leaf area: eenheden bladoppervlak per eenheid blad drooggewicht) en het aandeel van het plant drooggewicht dat in de bladeren is gelokaliseerd (LMF, leaf mass fraction). In formule:

$$RGR = NAR \bullet LAR = NAR \bullet SLA \bullet LMF$$

In hoofdstuk 3 is in een collectie van wilde *Capsicum* herkomsten en landrassen de invloed van verlaagde temperatuur op de RGR en groeigerelateerde eigenschappen (LAR en NAR) onderzocht. Hiertoe is een groeianalyse uitgevoerd bij twee temperatuurregimes (21.1°/18.7°C en 17.3°/14.7°C dag/nacht). Het relatieve verschil in RGR tussen planten die zijn opgegroeid onder deze verschillende temperatuurregimes is hierbij gebruikt als maat voor de aanpassing aan lagere temperaturen. Met dit relatieve verschil in RGR als maat bleken sommige *Capsicum* herkomsten beter te zijn aangepast aan lagere temperaturen dan anderen. Dit duidt er op dat er mogelijkheden zijn voor veredeling op lagere temperatuur tolerantie bij paprika.

De NAR was bij beide temperatuurbehandelingen de belangrijkste verklarende eigenschap voor genetische verschillen in RGR. De herkomsten die het beste waren aangepast aan lagere temperaturen hadden bij deze lagere temperaturen zowel een relatief hoge NAR als een relatief hoge LAR. In Hoofdstuk 6 is bediscussieerd dat het, ondanks het feit dat er bij veel genotypen een negatieve correlatie tussen NAR en LAR bestaat, mogelijk is een hoge NAR met een hoge LAR te combineren, resulterend in genotypen met een verhoogde RGR.

Hoewel RGR een belangrijke eigenschap is gebleken om variatie in koudetolerantie op te sporen, wordt deze eigenschap in de veredeling weinig gebruikt, voornamelijk omdat bepaling ervan zeer kostbaar is. Om deze reden is in Hoofdstuk 4 gezocht naar eenvoudig meetbare kenmerken die kunnen worden gebruikt als maat voor RGR. De correlatie tussen RGR en een aantal fysiologische en morfologische eigenschappen (relatieve groeisnelheid van het bladoppervlakte (RLGR), vers– en drooggewichten van bladeren en stengels, bladoppervlakte, planthoogte) werden bepaald in zowel de collectie van wilde *Capsicum* herkomsten en landrassen als in een groep paprikarassen. Met uitzondering van de relatie tussen RGR en RLGR konden deze relaties wel worden aangetoond in de *Capsicum* collectie maar niet in de groep paprikarassen. Om deze reden kunnen deze eigenschappen maar beperkt worden gebruikt als maat voor RGR. Daar tegenover stond dat RGR en RLGR wél sterk gecorreleerd waren, ook in groepen waar de variatie klein was.

Daarbij komt dat RLGR kan worden bepaald op basis van niet-destructieve bladoppervlaktemetingen, zoals beschreven in Hoofdstuk 2. Dit alles tezamen maakt RLGR een waardevolle, makkelijk te meten maat voor RGR (Hoofdstuk 6).

Verder is in Hoofdstuk 4 de te verwachten variatie voor lagere temperatuur tolerantie in de collectie van wilde *Capsicum* herkomsten en landrassen vergeleken met die in een groep paprikarassen. Binnen de collectie bleek deze variatie groter te zijn dan binnen de groep van paprikarassen. Op basis hiervan is geconcludeerd dat vooral wilde *Capsicum* soorten een waardevolle bron kunnen zijn van lagere temperatuur tolerantie waarbinnen veredelaars zouden moeten zoeken naar genetisch variatie voor deze eigenschap.

Om de fysiologische en morfologische kennis met betrekking tot lagere temperatuur tolerantie toepasbaar te maken voor de veredeling, is in Hoofdstuk 5 de overerving van RLGR en een aantal morfologische eigenschappen onderzocht. Dit is gedaan in een intraspecifieke *C. annuum* F_2 populatie met behulp van QTL analyse. QTL staat voor Quantitative Trait Locus. Dit is een gebiedje op een chromosoom (doorgaans gemarkeerd door één of meerdere DNA merkers) waarin zeer waarschijnlijk één of meerdere genen liggen voor een bepaalde eigenschap. De belangrijkste eigenschap waarvoor een QTL kon worden geïdentificeerd in de F_2 populatie was RLGR; dit QTL kon worden gevalideerd in een groep van geselecteerde F_3 lijnen. Daarnaast zijn 14 QTLs voor andere eigenschappen gerelateerd aan groei en ontwikkeling geïdentificeerd; zes hiervan konden worden gevalideerd in een groep van geselecteerde F_3 lijnen (o.a. die voor plant- en blad-drooggewicht, LMF en aantal bladeren). Omdat deze eigenschappen binnen de groep van paprikarassen niet zijn gecorreleerd aan RGR (Hoofdstuk 4), is de waarde van deze QTLs met betrekking tot groei ook beperkt; ze zijn waarschijnlijk wel belangrijk voor de plantontwikkeling. In Hoofdstuk 5 is aanmerkelijk gemaakt dat merker gestuurde selectie kan worden gebruikt bij de veredeling van paprika op verbeterde groei en ontwikkeling.

Samenvattend: In dit proefschrift is nieuwe kennis vergaard en zijn nieuwe methoden gepresenteerd die kunnen bijdragen aan veredelingsprogramma's welke erop zijn gericht paprikacultivars te

ontwikkelen die beter zijn aangepast aan lagere temperaturen. Hierdoor kan via veredeling een bijdrage worden geleverd aan de vermindering van zowel het energieverbruik als de CO_2 uitstoot in de glastuinbouw.

Nawoord

Eindelijk is het proefschrift af! Een moment van grote vreugde voor mij. Na ruim zeven jaar is het dan toch gelukt dit project tot een goed einde te brengen. Aan de andere kant is het ook een moment van verdriet vanwege het recentelijk overlijden van mijn moeder. Ondanks haar slopende ziekte had ze graag mijn promotie nog meegemaakt; ze overleed daags nadat ik haar de leesversie van dit proefschrift had gegeven. Mam, bedankt voor alles wat je voor me hebt gedaan en wat je me hebt meegegeven en voor je liefde, steun en geloof in mij. Ik draag het proefschrift aan je op als eeuwige dank voor alles.

Het doen van onderzoek en het schrijven van een proefschrift doe je niet alleen. Het is een proces waarbij vele mensen betrokken zijn. Ik wil hierbij dan ook iedereen van harte bedanken die heeft bijgedragen in de totstandkoming van dit proefschrift. Familie, vrienden en collega's: Bedankt! Enkele van jullie wil ik in het bijzonder bedanken.

In de eerste plaats wil ik Roeland, Leo en Piet bedanken voor het jarenlang begeleiden van mijn onderzoek en, de laatste jaren, bij het schrijven van dit boekje. Ik heb veel geleerd van jullie kritische kijk op mijn ideeën en manuscripten en van het enthousiasme waarmee jullie me hebben begeleid, ondanks de soms stevige discussies. Zelfs nadat ik uit Wageningen weg was en inmiddels ergens anders werkte, kon ik nog altijd op jullie rekenen. Dit was voor mij een stok achter de deur en belangrijke motivatie om dit project tot op een goede manier af te ronden.

Voordat je aan schrijven toekomt, is er reeds een traject van experimenteren en meten aan voorafgegaan. En ook het uitvoeren van experimenten kun je niet alleen. Experimenten die op papier nog te overzien zijn blijken in de praktijk ineens veel arbeidsintensiever dan gedacht. Maar ook bijkomstige zaken als het maken van kruisingen en schonen van zaden kosten altijd meer tijd dan gedacht. Ik heb het voorrecht gehad gedurende mijn hele project samen te mogen werken met Remmelt. Remmelt, ik wil je enorm bedanken voor de fijne samenwerking en al het werk dat je voor mij hebt gedaan. Naast een kundige collega, was je een leuke en inspirerende persoon. Zonder jouw enorme hulp en inzet zouden de experimenten heel wat minder resultaat hebben opgeleverd. Verder wil ik Jos bedanken die mij bij

vele experimenten heeft bijgestaan en mij jarenlang van verse honing heeft voorzien en natuurlijk al die anderen die bij tijd en wijle samen met mij metingen hebben verricht.

Mijn dank gaat verder ook nog uit naar de gewasverzorgers bij PRI en Unifarm. Ad wil ik in het bijzonder bedanken voor de zorg en toewijding waardoor ik altijd experimenten heb kunnen doen met top materiaal en voor het meedenken over de praktische kant van proeven en het oplossen van moeilijkheden.

Last but not least, wil ik Britta bedanken. Lieve Britta, het leven van een partner van een promovendus gaat (ook) niet altijd over rozen. Je wordt geconfronteerd met iemand die met perioden meer met z'n werk is getrouwd dan met z'n partner. En dan ook nog op de meest onwaarschijnlijke momenten vraagt om even wat door te lezen en een mening erover te geven. Voor al deze dingen heb je altijd veel begrip opgebracht en je hebt me altijd gesteund tijdens mijn promotie. Vaak wist je mij op een bijzondere manier een spiegel voor te houden waardoor ik weer inzicht kreeg waarom ik iets deed of weer vooruit kon in mijn denkprocessen. Ik wil je dan ook bedanken voor je steun en vertrouwen en voor de ruimte die je me hebt gegeven om dit project op mijn manier te doen. Zonder jou was ik er denk ik niet doorheen gekomen. Nu het af is hoop ik dat we weer wat meer tijd krijgen om samen met elkaar van de mooie dingen des levens te genieten.

List of publications

DE SWART, E. A. M., MARCELIS, L. F. M. and VOORRIPS, R. E. (2006). Variation in relative growth rate and growth traits in wild and cultivated *Capsicum* accessions grown under different temperatures. *Journal of Horticultural Science & Biotechnology*, **81**, 1029-1037.

DE SWART, E. A. M., GROENWOLD, R., KANNE, H. J., STAM, P., MARCELIS, L. F. M. and VOORRIPS, R. E. (2004). Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. *Journal of Horticultural Science & Biotechnology*, **79,** 764-770.

DE SWART, E. A. M., MARCELIS, L. F. M. and VOORRIPS, R. E. (2004). Search for low temperature tolerant *Capsicum* accessions to conserve energy in greenhouse pepper production. Proceedings of the XIIth Meeting on genetics and Breeding of *Capsicum* and Eggplant. (Voorrips, R. E., Ed.).101-107.

VAN WEES, S. C. M., DE SWART, E. A. M., VAN PELT, J. A., VAN LOON, L. C. and PIETERSE, C. M. J. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 8711–8716.

DE SWART, E. A. M., GROENWOLD, R., STAM, P. and VOORRIPS, R. E. QTLs for growth and growth related traits in *Capsicum annuum* L. *Plant Breeding, Accepted with revision*.

DE SWART, E. A. M., STAM, P., VOORRIPS, R. E. and MARCELIS, L. F. M. Influence of temperature on morphological plant traits and their relationship to relative growth rate in sweet pepper (*Capsicum annuum* L.) compared to a group of wild and cultivated *Capsicum* accessions. *Submitted*.

Curriculum vitae

Erik Augustinus Maria de Swart werd op 24 oktober 1973 geboren te Tegelen. In 1993 behaalde hij het VWO diploma aan het St. Thomascollege te Venlo en in hetzelfde jaar begon hij aan zijn studie Biologie aan de Universiteit Utrecht. Tijdens de doctoraalfase deed hij drie onderzoeken. Het eerste onderzoek werd, in samenwerking met de vakgroep Plantenfysiologie, uitgevoerd bij het Centrum voor Plantenveredelings- en Reproduktieonderzoek (CPRO-DLO) te Wageningen (onderzoek aan chrysant naar de mogelijkheden van genetische modificatie). Het tweede onderzoek werd uitgevoerd bij de vakgroep Fytopathologie (onderzoek naar de combinatie van twee vormen van ziekteresistentie in *Arabidopsis*). Het derde onderzoek werd uitgevoerd bij de vakgroep Ecofysiologie van Planten (onderzoek naar groei-eigenschappen van wilde gerst herkomsten). Zijn afstudeerscriptie schreef hij bij de vakgroep Fytopathologie (vergelijk van geprogrammeerde celdood in dierlijke en plantaardige systemen). In maart 1999 studeerde hij af en in april 2000 begon hij als assistent in opleiding bij de vakgroep Plantenveredeling aan Wageningen Universiteit. Het onderzoek werd uitgevoerd bij Plant Research International in nauwe samenwerking met de vakgroep Plantenveredeling. De resultaten van het onderzoek naar de mogelijkheid om de lage temperatuurtolerantie van paprika te verhogen middels veredeling staan beschreven in dit proefschrift. Gedurende het onderzoek is hij actief geweest binnen de onderzoekschool Production Ecology & Resource Conservation als lid van het PhD-students platform en als studentlid van zowel de Onderwijscommissie als de Onderzoekscommissie. Momenteel is hij werkzaam als beleidsmedewerker bij het gebied Aard- en Levenswetenschappen van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

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

Printed at Gildeprint Drukkerijen, Enschede.

Front cover:
Sweet pepper plant (*Capsicum annuum* L.)

Back cover:
Capsicum baccatum ‘PI 585241’ (top row)
Capsicum chinense ‘PI 543184’ (middle row)
Capsicum pubescens ‘PI 585265’ (bottom row)