A physiological and genetic analysis of growth characteristics in *Hordeum spontaneum*

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A physiological and genetic analysis of growth characteristics in *Hordeum spontaneum*

Een fysiologische en genetische analyse van groei-eigenschappen in *Hordeum spontaneum*

(met een samenvatting in het Nederlands)

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Contents

Chapter 1

General Introduction

Plant growth, in terms of the absolute increase in plant biomass or plant height per unit of time, has been of interest especially in agricultural context. Blackman (1919) was the first to recognise that the increase in plant mass is proportional to the plant biomass already present. The relative growth rate (RGR) is described as the rate of increase in plant mass per unit plant mass already present. RGR is an inherent quantitative trait that differs among plant species that occur in a wide range of habitats. Plants that occur in fertile habitats usually have a higher RGR than plants from nutrient-poor environments (Grime & Hunt 1975; Lambers & Poorter 1992). Even when plants are grown under close to optimal conditions, species from naturally less favourable habitats still have a lower growth rate than plants from fertile habitats (Grime & Hunt 1975; Lambers & Dijkstra 1987; Poorter & Remkes 1990; Garnier 1992; Atkin et al. 1996). RGR per se has probably not been the target of natural selection, but traits linked with or which underlie the RGR might have been selected for (Lambers & Poorter 1992; Poorter & Garnier 1999). Many studies try to explain the causes of inherent variation in RGR and the ecological consequences of differences in RGR. Differences in RGR can by explained be differences in leaf area per unit plant mass (LAR; leaf area ratio) or by differences in the rate of increase in plant mass per unit leaf area (ULR; unit leaf rate; (Evans 1972)). LAR is the product of the specific leaf area (SLA, total leaf area per unit leaf mass) and the leaf mass fraction (LMF, total leaf mass per unit total plant mass).

$$\mathrm{RGR} = \mathrm{ULR} \times \mathrm{SLA} \times \mathrm{LMF} \tag{1.1}$$

ULR is a more complex trait that represents the carbon gain in photosynthesis minus the carbon use in shoot- and root respiration, also taking into account the carbon concentration of the plant's newly formed biomass (Poorter 1989). In formula Poorter

(2002):

$$\mathrm{ULR} = \frac{\mathrm{PS_A} \times \mathrm{FCI}}{[\mathrm{C}]} \tag{1.2}$$

The first component in the right hand part of the equation is the rate of whole plant photosynthesis per unit leaf area (PS_A), integrated over the day. The second component is the fraction of daily fixed carbon that remains in the plant and is not respired or exuded by the various organs of the plants (FCI). The third component is the carbon concentration of the plant ([C]).

SLA is often considered as the prime factor associated with inherent variation in RGR (for a review see Poorter & van de Werf (1998)). SLA differs among plant species and is also affected by nutrient and/or moisture availability (Mooney et al. 1978; Shaver 1983), light intensity (Björkman 1981), temperature (Ku & Hunt 1973) or altitude (Körner 1989; Atkin et al. 1996). Plants with a low SLA have a lower amount of leaf area available for light capture and photosynthetic carbon gain and therefore generally have a low RGR. SLA is the reflection of leaf mass density and leaf thickness (Witkowski & Lamont 1991); the former is more often found to explain differences in SLA in herbaceous species (Garnier & Laurent 1994a; Van Arendonk & Poorter 1994) but in woody species leaf thickness seems to be more important (Niinemets 1999).

Dicotyledonous species with a low biomass allocation to the leaves may have a low RGR (Poorter & Remkes 1990), but this relationship is not found for grass species (Garnier 1991).

In most studies ULR is found to be of secondary importance to explain inherent differences in RGR (for a review see Poorter & Evans (1998b)). However a positive correlation can be found between ULR and RGR (Eagles 1967; Pons 1977); generally when shade species are compared with sun species under high-light conditions. Photosynthetic rate is the most important trait explaining differences in ULR and is, in general, strongly positively correlated with ULR (Konings 1989; Evans 1998; Poorter & van de Werf 1998). No correlation is found between photosynthetic rate per unit leaf area and RGR in most studies (Mooney et al. 1978; Delucia et al. 1989; Poorter 1989). Expressed per unit dry mass, however, photosynthetic rate is positively correlated with RGR. Plants with a high RGR usually also have a high SLA, so that their leaf biomass is spread over a larger area. Since they tend to have a similar light capture and photosynthetic carbon gain per unit leaf area as those plants with a low RGR, their carbon gain per unit leaf biomass is greater (Dijkstra & Lambers 1989b; Poorter et al. 1990). A positive correlation is usually observed between net assimilation rate of CO_2 and the nitrogen content per unit leaf area (Field & Mooney 1986; Evans 1989a). This may be explained by the fact that up to 75% of the leaf organic nitrogen is in the chloroplasts, most of it in the photosynthetic machinery (Evans & Seemann 1989).

Despite the strong correlation between photosynthesis and nitrogen the ratio between the rate of photosynthesis and the amount of organic nitrogen in the leaf, the photosynthetic nitrogen-use efficiency (PNUE), is not the same for all species (Field & Mooney 1986). Interspecific variation in PNUE can be explained, at low irradiance, by the fact that plants with high SLA and high PNUE have a lower organic nitrogen content per unit leaf area. At high irradiance, plants with a high PNUE allocate relatively more nitrogen to Rubisco, which tends to show a higher catalytic activity (Poorter & Evans 1998b). Two other components of the ULR are shoot and root respiration. The rates of shoot and root respiration often increase with an increasing RGR, due to the fact that plants need a faster respiration rate to support their faster growth (Van der Werf et al. 1988). Since fast- growing species have a higher organic nitrogen concentration, they may also have faster rate of maintenance respiration (Penning de Vries 1975). A third trait that is involved in the ULR is the carbon content. It was found that the carbon content does not play an important role in explaining interspecific differences in RGR, at least in herbaceous species (Garnier & Vancaeyzeele 1994b). However, sometimes a negative correlation between the two traits was found (Poorter & Bergkotte 1992a).

The advantage of having a high RGR could be a large biomass after a certain period of time, but this final mass also depends on the initial mass, which in turn is related to seed mass (Van Andel & Biere 1990; Alés et al. 1993; Marañón & Grubb 1993). The size of a seedling at any time after germination is, therefore, determined by both RGR and seed mass (Villar et al. 1998). The two traits may not be independent since very often a negative relationship is found between seed mass and RGR in interspecific studies across genera (Shipley & Peters 1990; Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998). In intraspecific studies this relationship is not found (Clevering 1999) or a positive relationship is found (Meerts & Garnier 1996).

The relationship of RGR with other growth traits might be different when taxonomic or functional groups are compared. It has been suggested that monocotyledonous species differ from dicotyledons in the importance of ULR (Garnier 1991; Van der Werf et al. 1998). In a comparison of annual and perennial grass species, annuals had higher RGR, ULR, LAR and SLA, and no differences in biomass allocation were found between annuals and perennials (Garnier 1992).

Several traits that are linked with or underlie RGR have been studied in great detail, but it still remains to be elucidated why exactly these correlations exist amongst the numerous traits mentioned above and a species' potential growth rate. One explanation could be that all these traits are controlled by a single common factor, such as the level of a certain hormone, e.g., gibberellin (Dijkstra & Kuiper 1989a; Dijkstra et al. 1990) or abscisic acid (Nagel et al. 1994). Alternatively, simultaneous selection for independent traits associated with a genotype's growth potential may have taken place. This problem cannot be solved on the basis of our present knowledge on differences

between species, but only by a detailed analysis of the variation between genotypes of a single species and crosses between them. Ideally this species should occupy a wide range of habitats and possess a large genetic variation. From numerous publications (Nevo et al. 1983; Nevo et al. 1984; Nevo et al. 1986; Corke et al. 1988; Nevo 1992) it emerged that *Hordeum spontaneum* C. Koch, the progenitor of cultivated barley (*Hordeum vulgare* L.) would be an ideal species in this respect. First I will report in this introduction a detailed description of the genus *Hordeum* and the variation that has been found in *Hordeum spontaneum* (C. Koch). Then I will report a description of QTL analysis and the use in plant physiology.

The genus *Hordeum* and *H. spontaneum*

The genus *Hordeum* belongs to the tribe Triticeae, in the grass family Poaceae. *Hordeum* comprises both annual and perennial species. The annuals are mainly inbreeding species whereas the perennials are very variable in their breeding systems. The majority of the wild, perennial species grow in rather moist environments like grassy meadows, lake shores etc. The annuals occur more often in open habitats, such as road sides with comparatively low competition (Von Bothmer & Jacobsen 1985). Some species are adapted to very special habitats, such as *H. comosum* that occurs in extremely dry steppe sides up to 4000 m in the Andes, *H. brevisubulatum* that grows up to 5000 m in the Himalayas or *H. bogdanii* that mainly occurs in saline environments (Von Bothmer & Jacobsen 1985). The native distribution of the genus is world-wide with one species (*H. capense*) in South Africa, and several species occurring in central and south-western Asia, western North America, southern South America and in the Mediterranean (Von Bothmer & Jacobsen 1985).

Cultivated barley (*H. vulgare*) is an important cereal crop species, ranking fourth in the world after rice, the wheats, and maize (Bengtsson 1992). Barley shows a wide range of adaptations to various environments and seems to be relatively well adapted to dry environments, where crops such as wheat fail (Whabi & Gregory 1989). About 10,000 years ago, somewhere in the Fertile Crescent, the ancient crop species barley was domesticated from its wild progenitor, *H. spontaneum* (Harlan & Zohary 1966; Zohary & Hopf 1988). Processes of domestication and selection have decreased the genetic variation of crop species (Tanksley & McCouch 1997). Therefore there is now an increasing interest in the wild progenitor species and primitive landraces of barley, as they may offer rich sources of genetic variation for crop improvement (Nevo 1992; Ceccarelli et al. 1995).

H. spontaneum, which is the subject of this thesis, is a convenient experimental organism because, (1) it is an annual with a short life cycle, (2) it is diploid with only seven pairs of chromosomes, (3) it is self-pollinating and (4) it exhibits variation in its

physiology, morphology and genetics. The genetic diversity of *H. spontaneum* has been studied, using isozyme polymorphisms, RFLP-markers (Saghai-Maroof et al. 1984), RAPD-markers (Dawson et al. 1993), SSR-markers (Saghai-Maroof et al. 1994) and AFLP-markers (Pakniyat et al. 1997), both within and amongst populations from the Fertile Crescent. These studies showed that *H. spontaneum* possesses more variation than cultivated barley, and that many alleles are associated with specific environments (reviewed by Nevo (1992), Forster et al. (2000)). A number of these studies found that most of the genetic variation was explained by differences between accessions within a population, rather than by differences between populations (Nevo et al. 1986; Dawson et al. 1993; Zhang et al. 1993; Baum et al. 1997). Variation in quantitative traits of agronomic importance has also been studied in *H. spontaneum*. This includes vegetative biomass and number and mass of spikelets, spikes and stems (Nevo et al. 1984) and yield and fecundity-related traits (Ivandic et al. 2001). Variation in physiological traits associated salt tolerance (Forster et al. 1997), drought tolerance and N-starvation (Robinson et al. 2000) has also been studied in *H. spontaneum*. In most of these studies variation in abiotic stress tolerance as well as genetic marker associations with plant traits and the site-of origin ecogeography were determined (Forster et al. 2000).

QTL mapping and marker association with plant traits

QTL is an acronym for Quantitative Trait Loci and has been described as genes that underlie quantitative traits (Gelderman 1975). Before 1980, the study of quantitative traits was based on statistical techniques, such as means, variances and covariances of relatives, with no knowledge of the number and location of the genes that underlie them. They were described by Mather (1949) as polygenes. Quantitative traits are traits that show a continuous range of variation in a population, which is more or less normally distributed (Kearsey 1998). Allelic differences that can occur in structural or regulatory genes, which alter the genes' action slightly, produce much smaller phenotypic effects and are assumed to underlie quantitative variation (Kearsey & Pooni 1996).

The development of molecular marker maps made it possible to construct dense genetic maps of a particular genome using appropriate mapping populations of plants (Quarrie 1996). Physiological traits, such as RGR and photosynthesis tend to be quantitative in nature (Prioul et al. 1997). Even though numerous loci may be involved in the expression of a physiological trait, the major-effect loci are expected to be quite few and to be detectable in a population of plants using molecular markers (Prioul et al. 1997). Three activities are required for QTL detection: (1) a mapping population of plants displaying genetic variability for the trait of interest, (2) construction of a ge-

netic map for that population by analysing the recombination ratios amongst molecular markers and (3) scoring the trait of interest on every individual of the mapping population (Prioul et al. 1997; Stam 1998). As a following step the genetic constitution of each line is compared to the mean trait value for each marker genotype at a particular locus (Quarrie 1996). When a significant difference between the genotype means is found, this indicates a probable linkage of the marker to a QTL nearby having an effect on the trait. Important for locating all the major QTLs for a particular trait and the accuracy with which they can be located is (1) the density and distribution of the markers on the genetic map, (2) the size and distribution of QTL effects, and (3) the size and type of mapping population (Quarrie 1996; Stam 1998). The segregating population has to exhibit phenotypic differences, which can be caused by phenotypic differences between the parental lines. Even when the differences between the parents are small or even nil, the theory of inheritance of complex traits predicts that the offspring may express a much larger variability, which is called transgression (Prioul et al. 1997).

The first QTL analysis on physiological traits in plants was performed in tomato where traits associated with fruit growth (mass, soluble solids content and pH) were mapped (Paterson et al. 1988). QTLs have subsequently been detected for physiological traits in more species of agronomic importance. In rice (*Oryza sativa*) stomatal conductance, leaf rolling (Price et al. 1997), seedling vigour traits (Redoña & Mackill 1996) and sodium and potassium uptake in determining salt tolerance (Koyama et al. 2001) were mapped. In maize (*Zea mays*) mostly drought stress-related traits and leaf ABA concentration were detected (Lebreton et al. 1995; Quarrie 1996; Tuberosa et al. 1998; Sanguinetti et al. 1999). Also in barley QTLs for several physiological traits, such as osmotic adjustment (Teulat et al. 1997), SLA (Yin et al. 1999b) and chlorophyll content (This et al. 2000) have been found (for a review see Forster et al. (2000)).

QTLs have also been detected for growth traits, like early vigour in soybean (Mian et al. 1998), early vigour in maize in relation to carbon metabolism (Causse et al. 1995), growth traits related to enzyme activity in maize (Prioul et al. 1999), tree growth related to architecture in poplar (Wu 1998). These studies had an agronomic background, and growth was measured as total plant height or leaf length. Until now no-one has mapped QTLs for growth being described as the relative rate of increase in biomass, or RGR. When QTLs are found for growth traits it would be interesting to search for candidate genes responsible for differences in growth traits, but then fine-mapping (Paterson et al. 1990) or construction of near-isogenic lines (Touzet et al. 1995) would be necessary to reduce the size of the chromosomal location of interest.

An alternative way of linking traits to molecular markers is a marker/trait regression analysis. This analysis provides an estimate of how well a marker is associated with the trait of interest. One of the major differences between marker/trait regres-

sion analysis and QTL analysis is that in the former regression is calculated between a trait and a single marker, which therefore limits the localisation of a chromosomal segment containing a QTL, whereas in the latter the QTL is located within a chromosomal interval, defined by flanking markers (Mauricio 2001). An advantage of association studies compared with QTL analysis is that no test of a segregating population is needed (which is expensive and time consuming), and the observed associations are not limited to a single cross (Kraakman et al. 2000). However, care has to be taken with the explanation of the observed associations, as the analysis can be imprecise and even misleading (Forster et al. 2000).

Outline of the thesis

In a first step the inherent variation in RGR and growth characteristics in the vegetative stage in *H. spontaneum* was determined (chapter 2). To this end 21 populations from different habitats in Israel were assessed for a suite of physiological, allocation-related, chemical and morphological growth characteristics. Also the relation of these growth traits with characteristics of the population's natural habitat was determined. Chapter 3 describes a comparison of the variation within *H. spontaneum* found in chapter 2 with the variation in growth traits in the genus *Hordeum*. Fifteen species of the genus *Hordeum*, including the *H. spontaneum* parents that were used in the subsequent cross, were subjected to a growth analysis to determine the relation of RGR with other growth traits and find differences between annual and perennial *Hordeum* species.

Chapter 4 forms the core of this thesis. The segregating population of a cross that was made between two contrasting *H. spontaneum* populations, was analysed for a range of growth related traits, and these traits were mapped on the genome with QTL analysis to determine if growth traits are genetically independent or caused by a common factor. Chapter 5 describes an association study of 70 markers with a suite of growth related traits in 21 previously analysed populations of *H. spontaneum*. Since these markers could be mapped on the same map used in chapter 4, we could analyse if the QTLs that were found before are only applicable to the specific cross we made, or if the QTLs are more general in wild populations of *H. spontaneum*. Finally, chapter 6 summarises and discusses the main results of chapters 2 to 5.

Chapter 2

Growth characteristics in *H. spontaneum* populations from different habitats

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Abstract

Hordeum spontaneum shows a large genetic variation and occupies a wide range of different habitats. The aim of this study was to quantify variation in growth characteristics of *H. spontaneum* from different sites in Israel and to relate this variation to different environmental conditions. To this end, 84 accessions of 21 populations were grown in a growth chamber in near-optimal conditions and a range of physiological, morphological, allocation-related and chemical characteristics were measured. These parameters included rates of photosynthesis, shoot and root respiration, specific leaf area, biomass allocation and seed mass. Averaged over all parameters variation explained by differences between populations was 26%, between accessions 21%, whereas that within accessions was 53%. By contrast with most genetic studies, we found variation be-

tween populations larger than between accessions. The largest between-population variation (46%) was for morphological traits. In particular, seed mass, leaf thickness and leaf width differed strongly between populations. Variation in growth characteristics between populations was poorly related to mean annual rainfall, mean humidity or January temperature at the sites of origin. We expect that differences between populations to be larger and correlation with environmental parameters stronger in plants grown in stressful conditions. According to our study, seed mass is more important than relative growth rate in determining variation in early plant biomass in *H. spontaneum*.

Introduction

Barley (*Hordeum vulgare*) is an important crop species often grown in areas with low rainfall, where other crops such as wheat fail (Whabi & Gregory 1989). The progenitor of this crop species is wild barley, *Hordeum spontaneum*, a diploid, self-pollinating annual, that harbours large genetic variation (Brown et al. 1978a; Nevo et al. 1979; Nevo et al. 1986; Corke et al. 1988; Dawson et al. 1993; Gunaskera et al. 1994; Petersen et al. 1994). As the rich gene pool of *H. spontaneum* can be used to improve the cultivated barley, variability in this species is of interest for plant breeders and geneticists. Because of its occurrence in a wide range of different habitats (Nevo et al. 1979), it is also interesting from an ecophysiological perspective.

Most research has focussed on genetic differences between populations of wild barley, using isozyme polymorphisms, RFLP-markers and RAPD-markers. Large variation has been found between and within populations of *H. spontaneum* from different sites in Israel. The variation in polymorphisms of allozymes and disease resistance can be related to their natural environment (Nevo et al. 1979; Nevo et al. 1984) also studied variation in quantitative traits of agronomic importance, such as vegetative biomass and number and mass of spikelets, spikes and stems. When grown under favourable conditions in a garden experiment, biomass of populations from mesic sites was about twice that of populations from xeric sites. It is therefore of interest to study the growth physiology of *H. spontaneum* and to investigate the causes of differences in biomass among plants from different habitats.

Differences in biomass can result from differences in seed mass, emergence time or variation in RGR (Van Andel & Biere 1990). Differences in RGR have been found among species from different habitats; those in favourable environments have an inherently high RGR, whereas those from less favourable habitats have an inherently low RGR, even when grown in the same favourable conditions (Grime & Hunt 1975; Poorter & Remkes 1990). This does not necessarily imply that RGR has been the target of natural selection. It might well be that characteristics that are linked with or

underlie RGR have been the selected for (Lambers & Poorter 1992; Poorter & Garnier 1999). Differences in environment such as rainfall, resource availability, altitude or temperature are correlated with RGR and its components (Lambers & Poorter 1992). In particular, Villar et al. (1998) found that *Aegilops* species from locations with a high annual rainfall have a higher RGR and invest less biomass in roots and more in shoots than species from drier locations.

Seed mass can also cause differences in biomass. In a study to determine the major factors that are responsible for variation in early vigour in barley, wheat and oat, embryo size was found the most important (López-Castañeda et al. 1996). Jurado & Westoby (1992) concluded that, among 28 native species from Central-Australia, seed size is more important than RGR or germination rate in determining seedling size 10 days after imbibition.

A comparison of strongly contrasting species has the advantage that differences in RGR and environment are generally large, but further genetic analysis is impossible. Thus we chose to work with a single species occupying different habitats, concentrating on genetic differences in growth and growth components in favourable conditions as part of a larger study of the relationships between genetics and growth. We compared 21 different populations of *Hordeum spontaneum* from Israel, and four accessions (seed families) from each population. We asked the following questions:

- To what extent do the growth characteristics of these populations differ?
- How much of the variation can be explained by differences between populations, between accessions and within accessions?
- Do populations that are environmentally related show similarity in growth characteristics?
- Do differences in rainfall, humidity or temperature explain inherent variation between populations?
- Are differences in vegetative plant biomass under nonlimiting conditions of water and nutrient supply caused by differences in seed mass or differences in maximum relative growth rate?

Material and Methods

Plant material and growth

We studied 21 wild barley (*Hordeum spontaneum* C. Koch) populations from several locations in Israel (Fig. 2.1) which represent a wide range of geographical and environmental conditions. The populations are listed in Table 2.1 with climatic data. For ease of reference we divided the populations, somewhat arbitrarily, into four ecogeographical groups: three Mediterranean (mountain (M), coastal plain (C) and steppic

(S)) and one from the desert (D). Three populations from Tabigha were sampled, two (Tab-TR and Tab-B) from a 100-m transect along an edaphic cline, one from terra rossa soil (derived from Middle Eocene hard limestone) one from basalt soils (generated on Pleistocene basalt flows, respectively (Nevo et al. 1983)). Two populations from Nahal Oren were sampled, one from the hot sunny south facing slope, one from the cooler more humid north facing slope (Nevo et al. 1997).

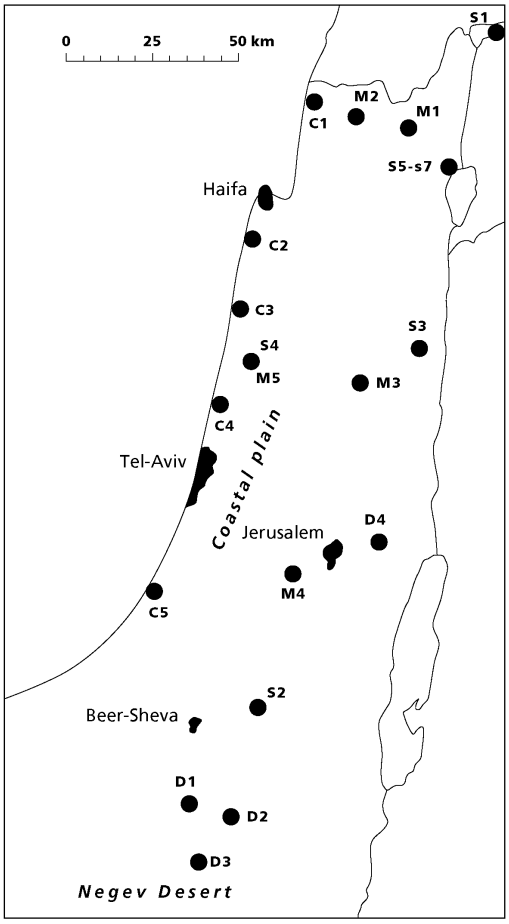

Figure 2.1: Geographic distribution of sampling localities of *Hordeum spontaneum* in Israel. The characters refer to the different populations in Israel, where M = mountain, C = coastal plain, S = steppic/marginal and D = desert.

Seeds were germinated on moistened filter paper in Petri dishes in a refrigerator at 6°C and an irradiance of $10\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$. After one week seedlings were transferred to a container with drainage holes which was filled with clean white beach sand. The sand was saturated with half strength of the following nutrient solution: $603\,\mu$M $Ca(NO_3)_2$, $795\,\mu$M KNO_3 , $190\,\mu$M KH_2PO_4, $270\,\mu$M $MgSO_4$, $0.2\,\mu$M $MnSO_4$, $0.9\,\mu$M $ZnSO_4$, $20\,\mu$M H_3BO_3, $0.3\,\mu$M Na_2MoO_4, $40\,\mu$M Fe-EDTA, $40\,\mu$M $FeSO_4$ and $47\,\mu$M SiO_2. The container was placed in a growth room for five days in the following conditions: 14/10 h day/night, 20°C day/night, irradiance of $450 \pm$

$25\ \mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$, relative humidity 70%. Thereafter seedlings were transferred to 33 L tanks containing the nutrient solution already described, aerated and at full-strength, which was replaced weekly. The pH of the nutrient solution was adjusted regularly to 5.8 with H_2SO_4. To avoid mutual shading, the number of plants on each container ranged from 18 and 6, depending on the size of the plants. Plants were rotated four times a week within the growth room.

Experimental design

Of each population, plants from four accessions (the progeny group from a single plant collected in the field) were grown. Of each accession eight plants were used to measure four sets of traits: allocation-related, physiological, chemical and morphological. Plants were measured at 23-25 days after germination, when there were 2-10 tillers. Whole-shoot photosynthesis and shoot and root respiration were measured on two plants of each accession. Fresh and dry mass of leaves, stems and roots, leaf area, leaf width, leaf angle, shoot height and the number of leaves and tillers were also determined on these two plants and on two additional plants. Two other plants were used for measurements of osmotic potential and to determine the chlorophyll concentration and the remaining two plants were used for measurement of leaf thickness. The latter four plants were also used for chemical analyses. Because of the large number of plants and the time needed for the physiological measurements we staggered the germination and growth of the plants. Four randomly chosen accessions of different populations were measured each week.

Measurements

Morphological traits Seeds were not dried before the experiment and contained approximately 6% water. Mass of each seed (coated caryopsis, without awn or spikelet stalk) was separately determined before germination with a Sartorius R160P balance. Leaf thickness and epidermal thickness were determined microscopically at five points at the middle of the youngest fully grown leaf: on the main vein, on the fourth vein to the left and right of the main vein and between the fourth and the fifth vein to the left and right of the main vein. Average leaf and epidermal thickness were calculated using these five points. Leaf width was taken as an average of measurements taken at five points in the middle of the youngest fully grown leaf. Leaf angle was determined by measuring the angle between the horizontal plane and the middle part of teach of the four oldest fully grown leaves; thus that of leaves with a vertical orientation $90°$.

Physiological traits Net photosynthesis and dark respiration were measured as CO_2 exchange. Intact plants were placed in a cuvette with shoots and roots in separate com-

No.	Site of origin	Longitude (°E)	Latitude (°N)	Altitude (m)	Mean temperature (°C)			Mean annual rainfall (mm)	Mean humidity at 14:00 hours (%)
					Annual	Aug	Jan		
Mediterranean									
	Mountain								
M_1	Mt. Meron	35.40	33.05	1150	14	22	6	1010	49
M_2	Maalot	35.27	33.00	500	17	23	8	790	50
M_3	Shechem	35.23	32.23	400	18	24	9	620	46
M_4	Bar-Giyyora	35.08	31.72	760	17	26	10	540	49
M_5	Nahal Oren (N)	35.02	32.43	75	19	24	11	690	59
	Coastal Plain								
C_1	Akhziv	35.10	33.05	10	20	26	12	620	60
C_2	Atlit	34.95	32.70	50	20	26	13	500	65
C_3	Caesarea	34.90	32.50	10	20	26	13	540	65
C_4	Herzliyya	34.80	32.17	25	20	26	13	530	65
C_5	Ashqelon	34.60	31.63	50	20	27	14	420	64
	Steppic / Marginal								
S_1	Mt. Hermon	35.75	33.28	1530	11	20	1	1600	52
S_2	Tel Shoket	34.92	31.32	375	19	26	11	280	45
S_3	Mehola	35.48	32.13	-150	22	30	13	270	34
S_4	Nahal Oren (S)	35.02	32.43	75	19	24	11	690	59
S_5	Tabigha	35.53	32.90	0	24	32	15	440	45
S_6	Tabigha (terra rossa)	35.53	32.90	0	24	32	15	440	45
S_7	Tabigha (basalt)	35.53	32.90	0	24	32	15	440	45
Desert									
D_1	Revivim	34.75	31.02	320	20	27	10	130	38
D_2	Yeroham	34.90	30.98	490	19	26	10	130	35
D_3	Sede Boqer	34.78	30.87	450	19	26	9	90	36
D_4	Wadi Qilt	35.38	31.83	50	23	30	14	170	40

Table 2.1: Location of the *Hordeum spontaneum* populations from Israel used for the analysis, and selected environmental data of sites of origin based on Nevo et al. (1984) and Pakniyat et al. (1997)

partments (Poorter & Welschen 1993). The root compartment was filled with a continuously aerated nutrient solution, similar to that supplied to the plants in the tanks. Irridiance was similar to that in the growth room. CO_2 and H_2O exchange were measured differentially with infrared gas analysers (ADC, model 225 MK3, Hoddesdon, UK), after equilibration for two hours. Calculations of all gas-exchange parameters were made according to Von Caemmerer & Farquhar (1981). In this way, whole-plant photosynthesis per unit leaf area (PS_A), per unit leaf mass (PS_M), shoot respiration (SR) and root respiration (RR) were assessed.

Allocation-related traits Total leaf area was determined using a Li-3100 area meter (Licor, Lincoln, NE, USA). Leaf area, fresh and dry mass of the leaves (leaf blades), stems (leaf sheaths) and roots were determined to calculate water content (fresh mass - dry mass / dry mass) of leaf, stem and root (WC_L , WC_S, WC_R, respectively), leaf area ratio (LAR, leaf area per total plant dry mass), specific leaf area (SLA, leaf area per leaf dry mass), leaf mass fraction (LMF, leaf dry mass per total plant dry mass), stem mass fraction (SMF, stem dry mass per total plant dry mass) and root mass fraction (RMF, root dry mass per total plant dry mass). Dry mass was measured after plant material had been dried for 48 hours at 70°C.

Chemical traits The osmotic potential was determined on leaf samples from the middle part (approximately 30-mm) of three leaves from one plant, which were stored in sealed plastic bags at -20°C. The osmotic potential of the leaf sap was measured using a Wescor (Logan, UT, USA) Vapour Pressure Osmometer, model 5100 C. The chlorophyll concentration of the leaf was determined according to Lichtenthaler & Wellburn (1983), after extraction with 80% acetone. To determine the concentration of C, total N, NO_3^-, organic N, organic acids and minerals, plants of each accession were combined into two independent samples. The C- and N-concentration of the samples were quantified with two elemental analysers (Carlo Erba 1106 and Carlo Erba 1110, Italy). Ash and ash alkalinity were determined as described by Poorter & Villar (1997). Results were used to calculate the organic acid and mineral concentrations. The nitrate concentration, quantified according to Cataldo et al. (1975) was subtracted from total N to determine the organic N concentration.

Calculations and statistical analyses Relative growth rate (RGR) was estimated, on the basis of allocation-related and physiological traits, using the formula given in Poorter & Pothmann (1992b):

$$\mathrm{RGR} = \frac{\mathrm{PS_A} \times \mathrm{SLA} \times \mathrm{LMF} - \mathrm{SR} \times (\mathrm{LMF} + \mathrm{SMF}) - \mathrm{RR} \times \mathrm{RMF}}{\mathrm{C}} \qquad (2.1)$$

(PS_A = Photosynthesis per unit leaf area, SLA= Specific Leaf Area, LMF =Leaf Mass Fraction, SMF = Stem Mass Fraction, SR = Shoot Respiration, RR = Root Respiration, RMF = Root Mass Fraction and C = Carbon concentration of the plant biomass). We only determined the carbon content of the leaves, assuming that it is representative of that of the whole plant. In reality, the carbon content of roots and shoots tend to be slightly lower than in the leaves (Poorter & Bergkotte 1992a), but these differences are not likely to affect RGR-calculation. A second assumption is that the rates of photosynthesis and respiration can be integrated over 24 hours. All parameters from the RGR-formula were measured at the same day. Data were analysed with SPSS for Windows (release 8.0; SPSS Inc., Chicago, IL, USA). Analyses of Variance (ANOVAs) were used to determine whether there were differences in the measured traits between populations and between accessions within populations. Variance components were calculated from the mean sum of squares, derived from a nested ANOVA (Sokal & Rohlf 1981). A discriminant analysis was carried out to separate the 21 populations. To compute the discriminant score all variables in the analysis were standardised. This means that, over all cases, the score from one function will have a mean of zero and a standard deviation of one. Relations between the various traits and the environmental data are described with linear multiple regression analyses.

Results

Variation between populations, between accessions and within accessions

To give an indication about the observed values of all the measured traits in *H. spontaneum* an overview of the average over all populations and the P10 (10th percentile), the P90 (90th percentile) values as well as the coefficient of variation for each trait is given in Table 2.2. This table also lists the percentage variation explained by differences between populations, between accessions and within accessions for each trait separately as well as the significance of the variance components. The variables are subdivided into four groups of growth characteristics; physiological traits and allocation-related traits listed in Table 2.2a, and chemical traits and morphological traits listed in Table 2.2b. The division of the variables is somewhat arbitrary, but are made to facilitate comparison of sets of traits. The coefficient of variation shows the variability of all the traits. For 23 out of the 31 traits, the coefficient of variation is lower than 20%. Circa 80% of the variation in RGR and photosynthesis per unit leaf mass is due to differences within accessions, whereas only 12% of the variation is explained by differences between populations. By contrast, 59% and 61% of the variation in morphological parameters like leaf width and leaf thickness respectively, is explained by differences between populations. Variation between populations was significant in all chemical and

morphological variables and almost all physiological and allocation-related variables. The between-accession variation was significant for almost all allocation-related traits and all chemical and morphological variables but not significant for most of the physiological traits. Focussing on the between-population and between-accession variation, most variables, particularly the physiological and morphological variables were found to have more variation between populations than between accessions (Tables 2.2a,b).

In summary, most of the variation in the measured traits was associated with differences within accessions rather than with differences between populations (Fig. 2.2a). This is particularly true for the physiological traits, where only 14% of the variation (Fig. 2.2b) is explained by differences between populations. The highest proportion of variance explained by differences between populations was observed for morphological traits (Fig. 2.2b).

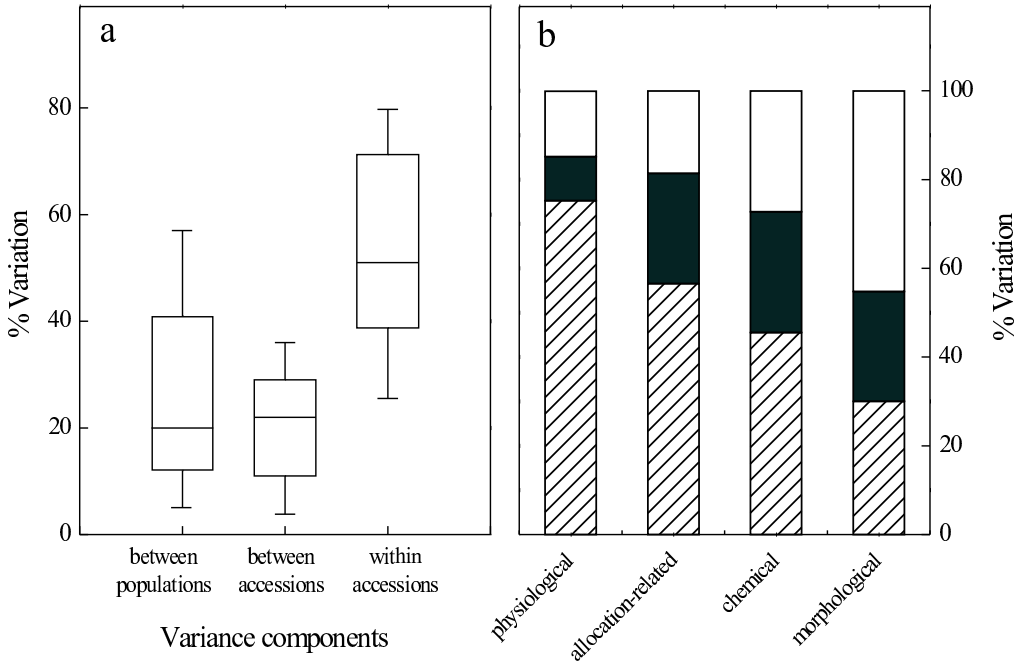

Figure 2.2: (a) Average percentage of variation explained by the variance components, for all 31 traits. The box shows 50% (median), the range of the 25% and 75% quartiles. The error bars show the 10% and 90% borders. (b) Percentage of total variation explained by the variance components (average values), for the four groups of measured traits.

Discriminant Analysis

The discriminant analysis in Fig. 2.3 separates the populations in Israel based on the variables related to a plant's carbon economy (RGR, PS_A, SR, RR, SLA, LMF, SMF,

Traits	Mean	P10	P90	Unit	Coefficient of variantion (%)	Variance components: Between populations (%)	Between accessions (%)	Within accessions (%)
Physiological								
Relative growth rate	300	250	350	mg g^{-1} day^{-1}	13	**12**	10	79
Photosynthesis per unit leaf area	15.4	13.3	17.7	μmol m^{-2} s^{-1}	12	**16**	12	72
Photosynthesis per unit leaf mass	550	510	600	nmol g^{-1} s^{-1}	9	**12**	8	80
Shoot respiration	47	39	53	nmol g^{-1} s^{-1}	12	6	15	78
% Respiration	40	33	48	%	17	5	**24**	71
Root respiration	65	44	86	nmol g^{-1} s^{-1}	28	**31**	0	69
Transpiration	1.64	1.20	2.09	mmol m^{-2} s^{-1}	26	**15**	0	85
Water-use efficiency	7.5	5.5	9.6	mg g^{-1}	21	**23**	10	67
Allocation-related								
Water content of leaf	6.9	6.0	7.7	g g^{-1}	10	**20**	**11**	70
Water content of stem	9.4	8.4	10.5	g g^{-1}	9	**20**	**30**	50
Water content of root	11.0	9.5	11.9	g g^{-1}	44	1	1	98
Leaf area ratio	16.7	14.3	19.3	m^2 kg^{-1}	12	9	**36**	55
Specific leaf area	35.2	30.3	40.7	m^2 kg^{-1}	11	5	**33**	62
Leaf mass fraction	0.47	0.44	0.51	g g^{-1}	6	**35**	**22**	43
Stem mass fraction	0.20	0.17	0.22	g g^{-1}	10	**18**	**36**	45
Root mass fraction	0.33	0.29	0.37	g g^{-1}	11	**41**	**29**	29

Percentages printed in bold indicate that the between accession or between population variation was significant in ANOVA ($P < 0.05$).

Table 2.2a: Mean, P10 (10^{th} percentile)-, P90 (90^{th} percentile)- values, units of allocation-related and physiological traits as well as the percentage of variation per trait explained by differences between populations (df=20), between accessions (df= 63) and within accessions (df= 84-252) for *Hordeum spontaneum* populations.

Traits	Mean	P10	P90	Unit	Coefficient of variantion (%)	Variance components: Between populations (%)	Between accessions (%)	Within accessions (%)
Chemical								
Chlorophyll content	430	350	520	μmol m^{-2}	13	**45**	**17**	38
Osmotic potential	-1.46	-1.65	-1.27	MPa	10	**18**	**17**	65
Mineral concentration	180	158	202	mg g^{-1}	10	**27**	**44**	28
Organic acid conc.	41	26	53	mg g^{-1}	23	**13**	**21**	66
Nitrogen conc.	65	60	69	mg g^{-1}	5	**28**	**28**	44
Carbon conc.	402	390	414	mg g^{-1}	3	**22**	**21**	57
Nitrate conc.	73.5	59.1	89.8	mg g^{-1}	18	**27**	**45**	28
Organic nitrogen conc.	48.1	43.7	51.5	mg g^{-1}	7	**38**	**11**	51
C:N ratio	6.2	5.8	6.7	g g^{-1}	6	**27**	**40**	33
Morphological								
Seed mass	36.3	18.8	48.4	mg	30	**53**	**11**	36
Leaf mass density	89	73	107	g mm^{-3}	17	**30**	**41**	29
Leaf angle	30.7	0	62.8	$^\circ$	76	**35**	**26**	39
Leaf thickness	340	270	400	μm	14	**61**	**10**	29
Leaf width	9.8	6.6	12.5	mm	23	**59**	**29**	11
Thickness of epidermis	66	54	76	μm	13	**36**	**15**	49

Percentages printed in bold indicate that the between accession or between population variation was significant in ANOVA ($P < 0.05$).

Table 2.2.b: Mean, P10 (10^{th} percentile)-, P90 (90^{th} percentile)- values, units of allocation-related and physiological traits as well as the percentage of variation per trait explained by differences between populations (df=20), between accessions (df= 63) and within accessions (df= 84-252) for *Hordeum spontaneum* populations.

RMF, C concentration) and three morphological variables, which differed the most between populations (seed mass, leaf thickness and leaf width). The independent variables were entered simultaneously. The first two functions explained 58% of the variation (Table 2.3). On the first function, seed mass was the most important variable discriminating between populations. On the second function, leaf thickness was the most important trait. Morphological traits were more variable on the two functions than variables related to a plant's carbon economy, and RGR was neutral (Table 2.3). Of the carbon economy traits, biomass allocation to roots and leaves were the most important. Populations with a small seed mass are situated on the left side of the graph (Fig. 2.3). These include most of the desert populations. Almost all of the mountain populations are on the right side of the graph. The coastal and steppic/marginal populations are scattered within the graph.

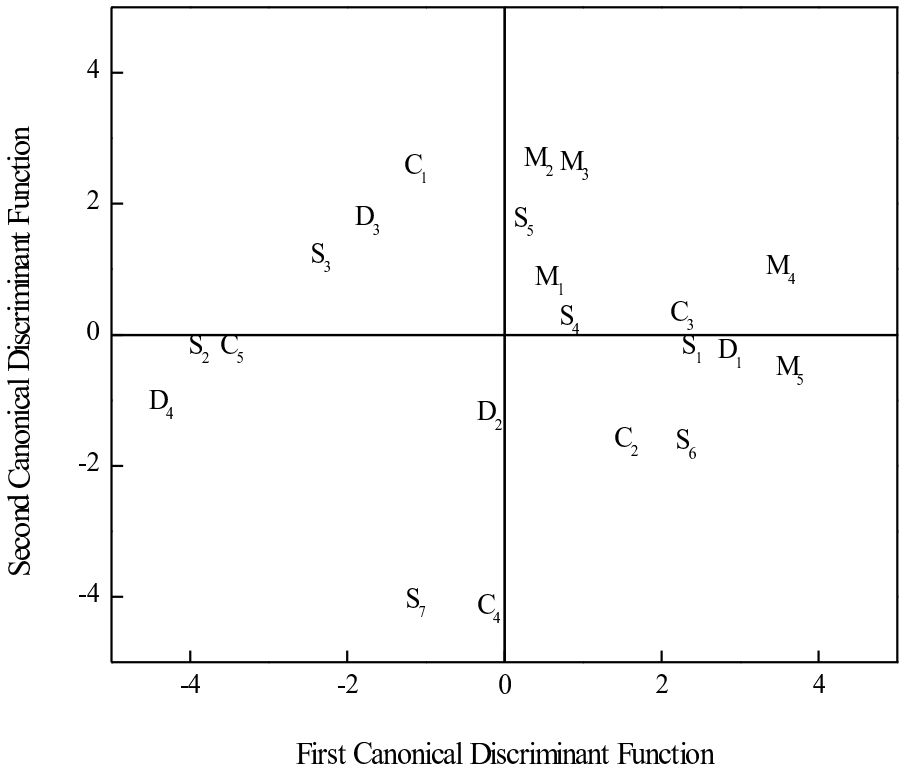

Figure 2.3: Discriminant analysis of variables from equation 2.1 (RGR, photosynthesis per unit leaf area, shoot respiration, root respiration, Leaf Mass Fraction, Stem Mass Fraction, Root Mass Fraction, Specific Leaf Area, Carbon content) and morphological variables (seed mass, leaf thickness, leaf width). The first discriminant function explaines 37% and the second discriminant function explaines 21% of the total variance. For explanation of characters see Table 2.1.

	Function	
Trait	1	2
Seed mass	0.66	-0.32
Leaf thickness	0.47	0.65
Root Mass Fraction	0.23	-0.10
Leaf width	0.18	0.52
Relative Growth Rate	0.05	-0.18
Photosynthesis per unit leaf area	0.03	-0.26
Shoot Respiration	0.01	-0.02
Specific Leaf Area	-0.07	0.03
Leaf Mass Fraction	-0.16	-0.03
Stem Mass Fraction	-0.18	0.19
Carbon Concentration	-0.23	-0.15
Root respiration	-0.37	-0.08

Table 2.3: Largest absolute correlation between each variable and any discriminant function.

Multiple regression between traits and environmental factors

Population S_1 (Mt. Hermon) was excluded from the multiple regression analysis between traits and environment, because this location not only has a very high rainfall but also a very high evaporation. Most of the investigated traits were not significantly correlated with an environmental factor at the site of origin. RGR was not related to any environmental factor (Table 2.4). PS_A was negatively correlated with rainfall and a positive relationship between SR and temperature was found. Water content of leaf and stem, as well as leaf width and leaf angle were positively correlated with rainfall. Seed mass had a positive relationship with humidity.

Seed size and final biomass

Seed mass varied by more than 200% between populations and total dry mass at harvest also by more than 200%, and the two were positively correlated (Fig. 2.4a). There was, on the other hand, no significant correlation between the estimated RGR of the various populations and their final biomass (Fig. 2.4b). Therefore, most of the variation in total biomass of these three and a half-week-old plants seems to be explained by variation in seed mass.

	Rainfall	Humidity	Temperature (Jan)	R^2
Relative growth rate	0	0	0	0.22
Photosynthesis per unit leaf area	-	0	0	0.32
Shoot Respiration	0	0	+	0.24
Water content of leaf	+	0	0	0.34
Water content of stem	+	0	0	0.34
Seed mass	0	+	0	0.40
Leaf width	++	0	0	0.57
Leaf angle	++	0	0	0.42

+, positive correlation; -, negative correlation; 0 for no significant correlation; $P<0.05$; $++$ or $--$ $P<0.01$

Table 2.4: Summary of multiple regression between traits (average per population) and environmental factors of 20 *Hordeum spontaneum* populations.

Discussion

Variation between populations, between accessions and within accessions

In this study we analysed the variation between and within populations of *H. spontaneum*, grown under favourable conditions. Calculated over all plants investigated, almost 75% of all traits had a coefficient of variation lower than 20%. We expected higher variation, taking into account that *H. spontaneum* is an inbreeder (Brown et al. 1978a). Interestingly, other intraspecific studies on morphological variation in inbreeding species (Wolff 1988; Bonin et al. 1997) report similar coefficients of variation. For further analysis, the variation in wild barley was divided into three variance components: between populations, between accessions and within accessions. Averaged over all parameters, variation within accessions explained 53% of the total variation. This is much higher than the values found for the variation between accessions within a population and between populations (21% and 26%, respectively). Maternal effects, environmental differences within the growth chamber or the influence of rare outcrossing rates (1.6% averaged over 26 populations; Brown et al. (1978b)), as well as stochastic factors could explain the large within-accession variation. The largest variation within accessions was observed for physiological traits and the smallest for morphological traits. A probable reason could be that the measurement errors involved in determining physiological variables are greater than for morphological variables.

In this study we calculated three variance components. However, when comparing these results with those from genetic variation studies in *H. spontaneum*, we have to consider that those studies often do not include the variation within accessions. This

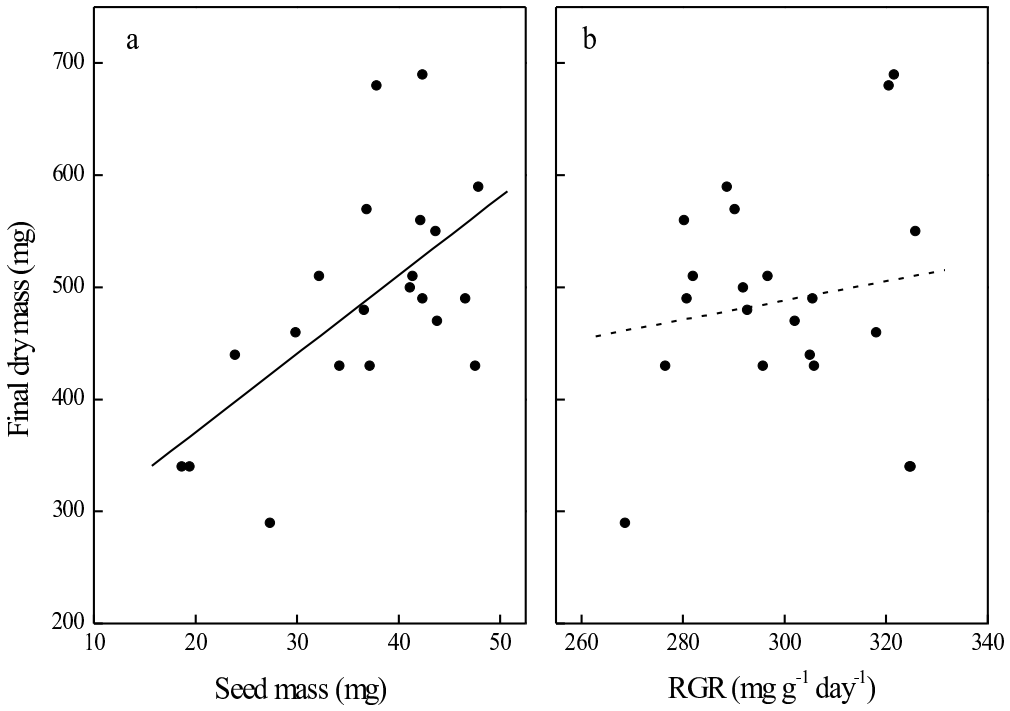

Figure 2.4: Relation between the final plant biomass and (a) seed mass (R^2=0.34) and (b) relative growth rate (R^2=0.02).

is because DNA is extracted from one plant only, or DNA samples are pooled, under the assumption that individuals within an accession are identical. This implies that the variation between accessions in those studies not only harbour the genetic variation, but also the variation within accessions. Table 2.2 shows that for most of the significant variance components, variation between populations is larger than within populations. This is contrary to many genetic diversity studies of allozymes and RFLPs in *H. spontaneum*, where most variation is explained by differences between accessions (Nevo et al. 1986; Dawson et al. 1993; Zhang et al. 1993; Baum et al. 1997). Most of these studies examined more accessions per population than our study, which might have resulted in a larger between-accession variation.

Photosynthesis expressed both per unit leaf area and per unit leaf mass (Table 2.2a), shows more variation between populations than between accessions, with the between-accession variation not significant. In contrast to these results, Carver & Nevo (1990) found in wild wheat (*Triticum dicoccoides*) populations that accessions within a given population showed 10-times more variation in PS_A or per unit chlorophyll than populations from different locations in a region. They concluded that genetic resources are located in relatively confined geographic regions.

Compared with the physiological and allocation-related variables, variation at the

population level in chemical and morphological traits is larger (Table 2.2b, Fig. 2.2b). For all traits in the last two groups the variation between populations as well as the variation between accessions is significant. The largest between-population variation in the morphological category was the variation in leaf thickness (61%) and in seed mass (53%). Corke et al. (1988) also found wide differences in chemical traits (nitrogen concentration in leaf and stem) as well as in allocation-related traits (leaf and stem mass) between seven populations of *H. spontaneum*. In the present study most of the variation (48%) in chemical traits is explained by differences within accessions. In conclusion: *H. spontaneum* populations show greater variation in morphological variables than in physiological variables.

Discriminant Analysis

The discriminant analysis (Fig 2.3) based on all the variables of equation 2.1 as well as seed mass, leaf thickness and leaf width, showed that the morphological traits were more important than the physiological or allocation-related traits in discriminating populations (Table 2.3). Seed mass was the most important trait loading on the first canonical discriminant function (Table 2.3), separating desert populations from mountain populations. Desert populations had on average lower seed mass. One desert population (D_1) is at the right side of the graph, being morphologically different from the other desert populations. It is possible that barley plants at this site (Revivim), in the North-western Negev desert which is characterised by a high amount of dew, are not exposed to extremely dry conditions. Van Groenendael (1985) reports in a within species (*Plantago lanceolata*) study, that plants from dry areas produce a large number of small seeds whereas plants from wet habitats produce fewer but bigger seeds. In contrast to these results are studies that make a comparison between species. Baker (1972) and Jurado & Westoby (1992) reported that species exposed to drought tend to have larger seeds and might therefore have larger root systems during early growth. On the second discriminant function (Table 2.3) leaf thickness was the most important trait. This function does not separate the populations very clearly, although it seems that most of the mountain populations have thicker leaves.

Multiple regression between traits and environmental factors

Most of the measured traits were not significantly correlated with an environmental factor at the site of origin. This might imply that the inherent differences between populations are not caused by adaptation to these environmental factors. However, one point of consideration is that the environmental data have not been measured directly at the sites of origin, but are averages from the nearest weather station. Another consideration is that we have grown plants under favourable conditions with an ample

supply of nutrients and water. Under stressful conditions, differences in physiology and relation to environmental factors will probably be larger. For example, Forster et al. (1997) found differences in experimentally determined abiotic stress responses (salt and drought tolerance) that are related to the environmental data of the sites of origin of *H. spontaneum* populations. The traits that showed a correlation with an environmental factor are listed in table 2.4. Plants from xeric environments have a higher rate of PS_A than plants from mesic areas. The same result was found by Nevo et al. (1991) in wild wheat (*T. dicoccoides*). In the present study, plants from a xeric climate have relatively narrow leaves, a more horizontal position of the leaves (a lower leaf angle) and more tillers. Plants from xeric climates generally have smaller leaves, which may help in reducing transpiration (Von Willert et al. 1992). By contrast, leaves in a more horizontal position have a higher light absorption and therefore a higher transpiration rate. The horizontal position might also explain the higher rate of PS_A in xeric populations. Surprisingly, SLA, leaf thickness or LMF, usually determinants of photosynthesis, did not correlate with any of the environmental factors. This is at variance with the suggestions of, for example, Poorter & Garnier (1999) and Ehleringer (1981) that plants in drier environments have lower SLA. For *Aegilops* species, Villar et al. (1998) did not find a relationship between SLA and rainfall, but they did find a relation between LMF and rainfall.

Seed size and final biomass

Nevo et al. (1984) found a higher biomass in mesic *H. spontaneum* populations than in xeric populations, grown in a mesic habitat. We obtained similar results for three and a half week old plants grown under near-optimal conditions. However, these differences in biomass are not caused by differences in RGR (Fig. 2.4b). Rather, differences in final mass could be explained by variation in seed mass (Fig. 2.4a). This is in agreement with Chapin et al. (1989), where seed size was found to be more important than RGR, after 35 days after sowing, in determining plant size in different *Hordeum* species under favourable nutrition. The xeric populations produce smaller seeds, but there was no correlation between seed mass and RGR. Seed size usually correlates negatively with RGR in interspecific studies (Shipley & Peters 1990; Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998). Meerts & Garnier (1996) studied seed size within a species (*Polygonum aviculare*) and found a positive relation between RGR and seed mass. Clevering (1999) studied also differences within a species (*Pragmites australis*), but found, like our study, no relation between RGR and seed size or between RGR and dry mass but a positive relation between seed size and dry mass. Similar suggestions were made by Van Andel & Biere (1990), Garnier & Freijsen (1994). In the present study, plants remained vegetative throughout the experiment. In

the field between-populations differences in reproductive effort or length of the growing season (no data available) can be additional factors that determine differences in biomass.

Conclusions

Under the present favourable growth conditions, *H. spontaneum* populations from Israel show a larger between-population variation than in most genetic studies. Most of the between-population variation occurred in morphological traits. The differences are not strongly related to the natural habitat, though xeric and mesic sites can be distinguished based on morphological traits. Seed mass is an important trait for which *H. spontaneum* has large variation and was more important than RGR in determining vegetative biomass in *Hordeum* in our study.

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Chapter 3

Variation in relative growth rate and growth traits in fifteen *Hordeum* species

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Abstract

We analysed relationships between relative growth rate, seed mass, biomass allocation and other growth-related traits in fifteen annual and perennial *Hordeum* L. species. Interspecific variation in RGR was only 30%, buffered by a strong negative correlation between unit leaf rate and specific leaf area. Due to this low variation, RGR was only related to LAR of its underlying growth parameters. Seed mass was more important in determining variation in vegetative biomass than RGR was. There was no difference in RGR between annual and perennial *Hordeum* species, but annuals had a larger seed mass, final biomass and unit leaf rate than perennials. The species could be separated in a Principal Component Analysis. For one of the species, *Hordeum spontaneum* (C. Koch), two populations were studied. Growth traits of a coastal population of *H. spontaneum* were similar to a cultivar of *H. vulgare* L. (Reggae), while growth traits of a steppic population were more similar to the other wild *Hordeum* species. Six *Hordeum* species were subjected to a more detailed growth analyses. According to this study, variation amongst *Hordeum* species in morphological growth traits is larger than in physiological growth traits. Variation in growth traits in *H. spontaneum* was on

average 99% of the total variation in *Hordeum* species.

Introduction

The genus *Hordeum* comprises 32 species and many of these occur in temperate areas in both the northern and the southern hemisphere. Some species inhabit subtropical areas in central South America and arctic habitats in North America and Central Asia and are found from sea level up to more than 4500 m in the Andes and the Himalayas (Von Bothmer et al. 1995). The majority of the *Hordeum* species are confined to grassland habitats. *Hordeum* comprises both annual and perennial species, which is not common in most genera in the Triticeae.

Differences amongst *Hordeum* species have been studied at various levels, including, genome relationships (Von Bothmer et al. 1987), protein relationships (Jørgensen 1986), morphological relationships (Von Bothmer & Jacobsen 1985) and disease resistance (Gustafsson & Claesson 1988). Only few have focused on variation in physiology and growth amongst *Hordeum* species. Chapin et al. (1989) studied physiological determinants of growth in four *Hordeum* species. We want to obtain a broader perspective of the genus *Hordeum* and therefore study physiological and morphological determinants of growth in fifteen *Hordeum* species.

Variation in plant vegetative biomass can be caused by differences in seed mass, emergence time or differences in relative growth rate (RGR, the increase in plant mass per unit mass present and per unit of time; Van Andel & Biere (1990)). The potential growth rate of plant species varies widely, for plants grown under 'close to optimal' conditions. RGR and its underlying components have been studied extensively in recent years (Lambers & Poorter 1992; Poorter & van de Werf 1998). Many studies analysed the causes of inherent variation in RGR and the ecological consequences of differences in RGR. Differences in RGR can be explained by differences in leaf area ratio (LAR, total leaf area per total unit plant mass) and/or unit leaf rate (ULR, rate of increase in plant mass per unit leaf area per unit of time). LAR is the product of the specific leaf area (SLA, total leaf area per unit leaf mass) and the leaf mass fraction (LMF, total leaf mass per unit total plant mass). ULR is a complex trait that represents the carbon gain in photosynthesis and the carbon losses in shoot- and root respiration and exudation as well as the carbon concentration of the plant. In an analysis of literature Poorter & van de Werf (1998) concluded that LAR and more specifically SLA is the most important factor explaining inherent variation in RGR. On the other side, some studies prove that ULR is more important determining differences in RGR (Eagles 1967; Pons 1977).

Seed mass is also very important in explaining variation in dry mass at an early vegetative stage (Chapin et al. 1989; Marañón & Grubb 1993; Van Rijn et al. 2000).

In a study of the major factors responsible for variation in early vigour in barley, wheat and oat, embryo size was found to be the most important (López-Castañeda et al. 1996). Sometimes RGR and seed mass are negatively correlated (Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998), sometimes there is no relationship (Shipley & Peters 1990; Van Rijn et al. 2000) and sometimes they are positively correlated (Meerts & Garnier 1996).

Differences in the relationship of RGR with other growth traits may occur when different taxonomic or functional groups are compared. For example, it has been suggested that monocots differ from dicots in the importance of ULR (Garnier 1991; Van der Werf et al. 1998). Garnier (1992) found a higher RGR and ULR in a growth analysis of congeneric annual than in perennial grass species. Until now most studies on RGR and growth-related traits have focused on differences between species across genera and only few studies have evaluated inherent growth variation within a genus (Chapin et al. 1989; Atkin et al. 1996; Meerts & Garnier 1996; Villar et al. 1998). For the genus *Hordeum*, growth characteristics were determined previously by Chapin et al. (1989), but due to the limited number of species in their study (four), it remains difficult to deduce general patterns and relationships between growth traits. The objectives of this study were: (1) to analyse the variation in RGR and growth-related traits in *Hordeum* species; (2) to examine the traits underlying variation in biomass; (3) to analyse the differences between annual and perennial *Hordeum* species.

In a previous paper we studied variation in growth-related traits within one species: *Hordeum spontaneum*, the progenitor of cultivated barley (Van Rijn et al. 2000). We showed that there was little variation in physiological and allocation-related traits, but large variation in morphological traits. In the present study, the genus-wide growth analysis enables us to compare growth-related traits of *H. spontaneum* with other wild *Hordeum* species. Hence, two additional objectives of this study are: (4) to asses if populations of *H. spontaneum* are more similar in their growth-related traits to other wild *Hordeum* species or more similar to *H. vulgare*, and (5) to compare interspecific variation in *Hordeum* species with the intraspecific variation as found earlier in *H. spontaneum* (Van Rijn et al. 2000). Therefore two populations of *H. spontaneum* (one from a mediterranean site and one from a steppic/marginal mediterranean site) were included in this study. These populations were crossed and the offspring of this cross was used in a forthcoming paper studying quantitative trait loci of growth-related traits in *H. spontaneum*. For the last question six *Hordeum* species from different continents were compared in a more extensive growth analysis, where the same traits were measured as in Van Rijn et al. (2000).

Material and Methods

Plant material and growth

Fifteen *Hordeum* species from habitats all over the world were studied. Seeds from thirteen *Hordeum* species were kindly provided by the Institute for Plant Genetics and Crop Plant Research (IPK Gatersleben). Two populations of *H. spontaneum* (C. Koch) of different locations in Israel were used, kindly provided by Prof. E. Nevo (Haifa University). One of these was a coastal population (Ashqelon) and the other (Mehola) was collected in the Jordan Valley, which is a more steppic, marginal mediterranean habitat (Nevo et al., 1983). *H. vulgare* (Reggae) seeds were provided by the Department of Plant Breeding (Wageningen Agricultural University). Table 3.1 shows a list of the species as well as the collection sites, life form, habitat description and accession numbers.

Seeds were sterilised for 5 minutes in 10% (v/v) of hypochloride and subsequently rinsed with water. Seeds were germinated on moistened filter paper in Petri dishes in a growth cabinet (day-14 h, 25°C, 30 μmol m^{-2} s^{-1} PAR; night-10 h, 15°C). After one week seedlings were transferred to a container with drainage holes, filled with clean white beach sand. The sand was saturated with half strength of the following nutrient solution: 603 μM $Ca(NO_3)_2$, 795 μM KNO_3 , 190 μM KH_2PO_4, 270 μM $MgSO_4$, 0.2 μM $MnSO_4$, 0.9 μM $ZnSO_4$, 20 μM H_3BO_3, 0.3 μM Na_2MoO_4, 40 μM Fe-EDTA, 40 μM $FeSO_4$ and 47 μM SiO_2. The container was placed in a growth room, under the following conditions: 14/10 h day/night, 20°C day/night, irradiance of 450 ± 25 μmol m^{-2} s^{-1}, relative humidity 70%. After five days seedlings were transferred to 33 L tanks containing a full-strength, aerated nutrient solution as described above, which was replaced weekly. This day was considered day 0. The pH of the nutrient solution was adjusted regularly to 5.8 with H_2SO_4. All plants remained vegetative during the experiment and none showed dead leaves.

Experiment 1: Growth analysis of 15 species

Before the seeds (coated caryopsis, without awn or spikelet stalk) were germinated, seed mass (air-dry) was determined as an average of 15 to 30 seeds. The growth experiment started when the plants were 14 days on nutrient solution. Harvests were carried out on day 14 and 21. The plants of the 15 species studied (listed in Table 3.1) were grown in the same growth chamber at the same time.

Per species and per time point 4 plants were harvested. The experiment was repeated two months later. Total leaf area was determined using a Li-3100 area meter (LI-COR, Lincoln, NE, USA). Leaves (leaf blades), stem (leaf sheaths) and root fresh and dry mass were determined with a Sartorius R160P (Göttingen, Germany). Data

	Species	Life-form	Origin	Habitat	Acc nr.
1	*H. bogdanii*	perennial	Pakistan	saline (shores of lakes, streams and ponds) and wet areas	GRA 969 / 88
2	*H. brachyantherum*	perennial	USA	salt marshes, pastures, woodland, subalpine meadows, up to 4000m	GRA 967 / 97
3	*H. brevisubulatum*	perennial	China	wide array of biotopes	GRA 1080 / 84
4	*H. bulbosum*	perennial	Greece	wet meadows, dry hillsides, roadsides	GRA 2617 / 97
5	*H. capense*	perennial	South Africa	fertile soil in wet places (river banks, mountain meadows)	GRA 971 / 87
6	*H. chilense*	perennial	Chile	pastures in mesic and xeric habitats	GRA 972 / 89
7	*H. comosum*	perennial	Argentina	dry habitats, steppe end dry hillside	GRA 2664 / 96
8	*H. cordobense*	perennial	Argentina	grasslands	GRA 974 / 97
9	*H. flexuosum*	perennial	Argentina	pastures, riverbeds, sometimes saline places	GRA 977 / 88
10	*H. intercedens*	annual	USA	often flooded areas (vernalpools in shrublands)	GRA 979 / 88
11	*H. marinum*	annual	Italy	saline meadows or marshes along coast or inland (sometimes as a weed)	GRA 1309 / 91
12	*H. murinum*	annual	Greece	different habitats, mainly as a weed	GRA 2619 / 95
13	*H. pusillum*	annual	USA	grassland borders of marshes, ruderal, often alkaline soil	GRA 987 / 92
14	*H. spontaneum* Mehola	annual	Israel		22-28
15	*H. spontaneum* Ashqelon	annual	Israel		28-77
16	*H. vulgare*	annual	-		-

Table 3.1: List of 15 *Hordeum* species studied, life-form, place of origin habitat description (pers. comm. R. Von Bothmer) and accession numbers.

were used to calculate RGR, unit leaf rate, leaf area ratio, specific leaf area, leaf mass fraction, stem mass fraction (SMF, total stem mass (including leaf sheath) per unit total plant mass and root mass fraction (RMF, total root mass per unit total plant mass).

Experiment 2: Growth-related traits of six species

Six *Hordeum* species (*H. brevisubulatum, H. capense, H. comosum, H. flexuosum, H. intercedens* and *H. marinum*) that differed in relative growth rate were subjected to a more extensive growth analysis. The measurements are described below and were done on 28- day old plants. The reason older plants were used, was that the increased biomass of the plants would enable better measurements of photosynthesis and respiration. Therefore another growth analysis was carried out with harvests on day 21 and day 28. Per species and per time point 4 plants were harvested. The same parameters were measured and calculated as described in 'Experiment 1'.

In addition four sets of traits were measured in 28-days-old plants: morphological traits, allocation-related traits, chemical traits and physiological traits. Each week two plants of four species were measured for a suite of traits. This was repeated for six weeks so that in total eight plants per species were measured. Four sets of traits were measured. Seed mass was determined as the average of 10 seeds. Leaf width, leaf thickness, epidermal thickness were measured on the youngest fully-grown leaf. Leaf angle was assessed as the average angle of the four oldest leaves. Plant height was determined by measuring total shoot length.

Total leaf area, fresh and dry mass of leaves, stems and roots were determined (as in experiment 1) to calculate: leaf area ratio, specific leaf area, leaf mass fraction, stem mass fraction, root mass fraction, water content of the leaves, stems and roots.

Carbon content and nitrogen content were determined with an elemental analyser (Carlo Erba 1110, Milan, Italy). Nitrate concentration was quantified according to Cataldo et al. (1975). Ash and ash alkalinity were determined as described by Poorter & Villar (1997). Results were used to calculate concentrations of organic acids, minerals and organic nitrogen compounds. The osmotic potential of the leaf sap was measured using a Wescor (Logan, UT, USA) Vapour Pressure Osmometer. The chlorophyll concentration of the leaf was determined according to Lichtenthaler & Wellburn (1983) after extraction with 80% acetone.

Net photosynthesis, dark respiration and root respiration were measured as CO_2 exchange. CO_2 and H_2O exchange were measured differentially to calculate photosynthesis per unit leaf area and per unit leaf mass, shoot respiration, root respiration and water use efficiency.

All traits were measured as described previously in Van Rijn et al. (2000).

Calculations and statistical analyses

Data were analysed with SPSS for Windows (release 8.0; SPSS Inc. Chicago, IL, USA). Relationships between growth and other variables across the fifteen species (n=16, because the two *H. spontaneum* populations were analysed as separate data points) were analysed by correlation and linear regression (type II) of species means. Analyses of Variance (ANOVAs) were used to determine whether there were differences in the measured traits between species. Variance components were calculated from the mean sum of squares, derived from an ANOVA (Sokal & Rohlf 1981). To separate the fifteen species, based on growth traits, a Principal Components Analysis (Splus 2000, Mathsoft, Inc. USA) was performed. Differences between life forms (annual-perennial) were tested using an independent sample T-test. The proportional difference in a particular growth parameter X is defined as GRC_X (Growth Response Coefficient; (Poorter & van de Werf 1998). In formula:

$$GRC_X = \frac{\frac{dX}{X}}{\frac{dRGR}{RGR}} \tag{3.1}$$

Full details on GRC and its calculation are described in Poorter & van de Werf (1998).

Results

Variation in RGR and growth related traits

RGR varied 30% amongst the fifteen species, ranging from 203 mg g^{-1} day^{-1} for *H. comosum* to 260 mg g^{-1} day^{-1} for *H. murinum* (Appendix 1). Species with a high RGR also had a high LAR ($P < 0.05$; Fig. 3.1B). RGR was not significantly correlated with any other trait (Fig. 3.1), except for an almost significant correlation with SLA (P=0.06; Fig. 3.1C). There was a strong negative correlation between ULR and LAR ($P < 0.01$; Fig. 3.2). The variation in LAR was mainly due to variation in SLA ($P < 0.01$) and to a lesser extent to variation in LMF ($0.05 < P < 0.10$). Therefore the strong negative correlation between ULR and LAR is due to the negative relation between ULR and SLA ($P < 0.05$). To compare this study with other studies of growth related traits the Growth Response Coefficients were calculated (Poorter & van de Werf 1998). GRC_X is defined as the proportional difference in a particular growth parameter X (ULR, LAR, SLA, LMF), scaled to the proportional difference in RGR (Poorter & van de Werf 1998). Table 3.2 shows the GRC-values of this study compared with the results in 20 *Aegilops* species (Villar et al. 1998) and with 11 grass species (Poorter & Remkes 1990). The GRC-values of the 16 *Hordeum* are much more comparable with the 11 grass species than the study between 20 *Aegilops* species.

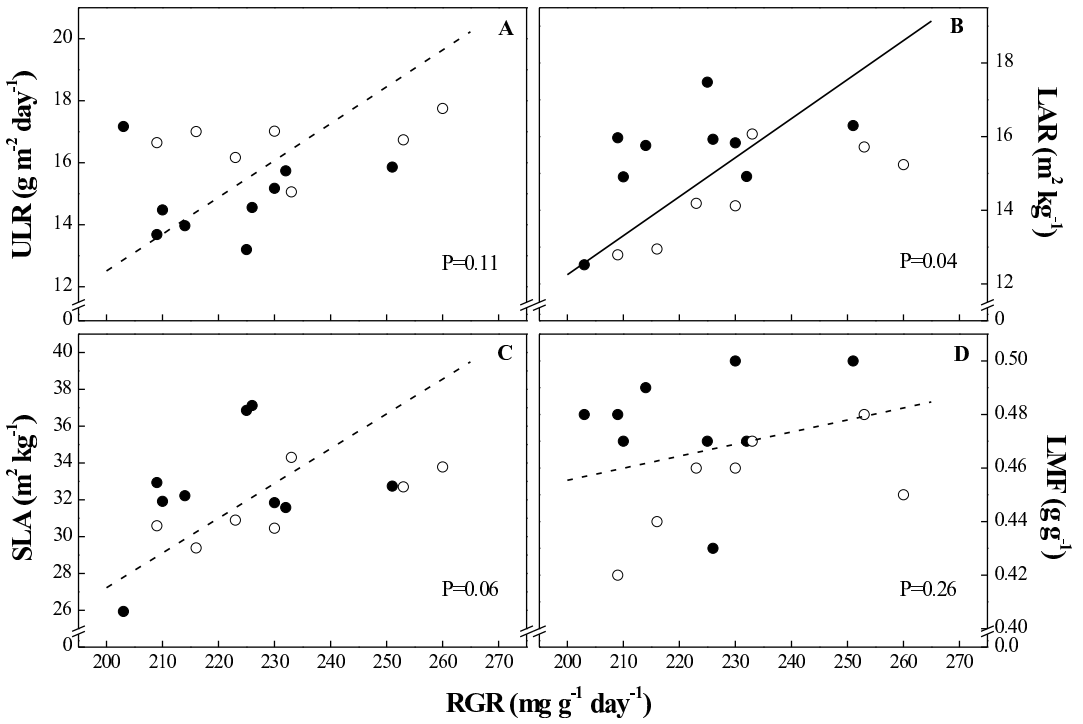

Figure 3.1: Relationship for the 15 *Hordeum* species between mean values of relative growth rate and (a) unit leaf rate, (b) leaf area ratio, (c) specific leaf area, (d) leaf mass fraction (○ annuals; • perennials)

What explains variation in dry mass?

The dry mass of the fifteen species at the end of the experiment (circa 28 days after germination) varied between 250 mg for *H. comosum* and 4380 mg for *H. spontaneum* (Ashqelon) (Fig. 3.3). To find out whether this difference was due to variation in RGR or to variation in seed mass, the two parameters were plotted against dry mass at day 21 (Fig. 3.3) A positive correlation between biomass at day 21 and seed mass (R= 0.91; $P < 0.001$) was found (Fig. 3.3A). There was, however, no significant correlation between the RGR and biomass at day 21 (Fig. 3.3B; $P > 0.1$). There was also no significant correlation between seed mass and RGR (Table 3.3).

Differences between annuals and perennials

There was no significant difference in RGR between annuals and perennials (Table 3.4). Annuals had a higher ULR than perennials (Fig. 3.1A; $P < 0.05$). There was no difference in LAR or SLA, but perennials had a higher LMF (Table 3.4). Annuals had a much higher seed mass and also higher final biomass (Fig. 3.2A, Table 3.3; $P <$

	15 *Hordeum* species	20 *Aegilops* species	11 Grass species
GRC_{ULR}	0.38	0.54	0.33
GRC_{LAR}	0.62	0.46	0.70
GRC_{SLA}	0.51	0.33	0.64
GRC_{LMF}	0.11	0.13	0.02

Table 3.2: GRC values of 15 *Hordeum* species, 20 *Aegilops* species (Villar et al. 1998) and 11 grass species (Poorter & Remkes 1990).

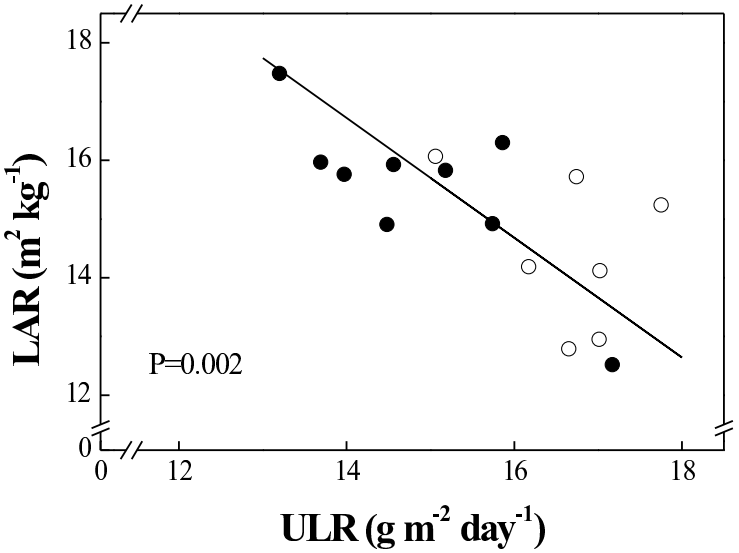

Figure 3.2: Relationship for the 15 *Hordeum* species between mean values of unit leaf rate and leaf area ratio (R = -0.69; $P < 0.01$). (○ annuals; • perennials)

0.05). The water content of the stem was also larger in annuals (Table 3.4).

Principal Component Analysis

Figure 3.4 shows a Principal Component Analysis of the fifteen *Hordeum* species based on growth characteristics (RGR, ULR, LAR, SLA, LMF, SMF, RMF, WCleaf, WCstem, WCroot and seed mass). The first component explained 58%, and the second component explained 40% of the variation. On the first component, RGR was the most important variable, and on the second component seed mass was the most dominant variable explaining variation in *Hordeum* species. The PCA shows four separate groups of species, where on the first axis on the right side of the graph species were situated with a high RGR and on the left side species with a low RGR. On the se-

Independent/ Dependent variable	Trait values	
	r	P
RGR		
RMF	-0.08	n.s.
Final dry mass	+0.31	n.s.
Seed mass	-0.07	n.s.
ULR		
LAR	-0.73	**
SLA	-0.61	*
Final dry mass	+0.47	$\sim$
LAR		
SLA	+0.85	**
LMF	+0.35	n.s.
Final dry mass		
Initial mass	+0.99	***
Seed mass	+0.79	***
Water content leaf	+0.68	**
Seed mass		
Initial mass	+0.87	***
Water content leaf	+0.57	*
SLA	-0.30	n.s.
LMF		
SMF	-0.50	*
RMF	-0.08	n.s.
RMF		
SMF	-0.79	***
Water content total plant	+0.53	*

Table 3.3: Summary of regression results for different variables in the 15 *Hordeum* species studied. Significance: $\sim$ $0.10 > P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant.

cond axis species with a high seed mass were in the upper part of the graph, and species with a low seed mass in the lower part. *H. vulgare* and the coastal population of *H. spontaneum* were clearly separated from the other *Hordeum* species. *H. spontaneum* (steppic) was grouped with *H. chilense* and *H. murinum*.

Comparison of variation in *Hordeum* species with variation in *H. spontaneum*

Six *Hordeum* species from five continents were subjected to a more extensive analysis of growth traits. To compare the variation of these growth traits within the genus *Hordeum* with the variation within *Hordeum spontaneum* (Van Rijn et al. 2000), the measured traits were divided in four groups. To compare the range of variation in

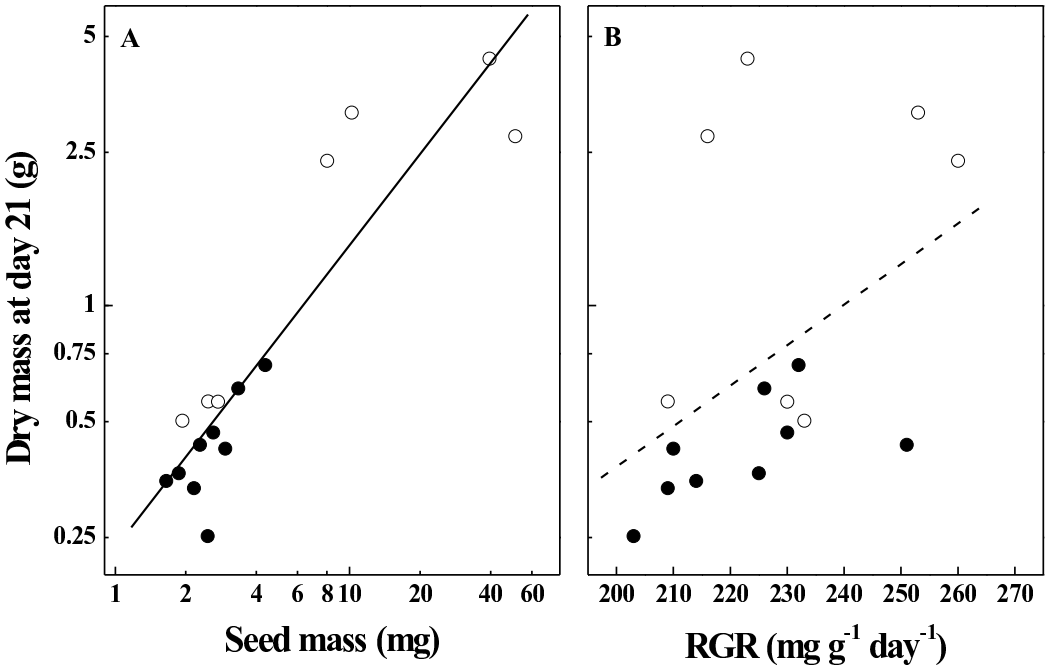

Figure 3.3: Relation for the 15 *Hordeum* species between mean values of the final plant biomass (log scale) and (a) seed mass (log scale) (R = 0.91) and (b) relative growth rate (R = 0.44). (○ annuals; ● perennials)

growth traits between the *Hordeum* species and the 21 populations of *H. spontaneum*, used in Van Rijn et al. (2000) total range values were calculated on the average values (per species or per population) of each trait. To normalise all the different trait values, each range was scaled to the highest value. For the allocation-related traits the data of experiment 1 (except for the *H. spontaneum* populations) were used to calculate range (n=14). For the physiological and morphological traits the data of experiment 2 plus data from *H. vulgare* (cultivar L94; data not shown) were used to calculate the range (n=7). For chemical traits data from experiment 2 were used. A larger bar length for the *Hordeum* species in Fig. 3.5 means that the variation in that specific trait is larger than the variation amongst 21 populations of *H. spontaneum*. The variation in all allocation-related traits (Fig. 3.5B; on average 62%) is for all traits larger amongst *Hordeum* species than amongst populations of *H. spontaneum*. This is also true for almost all morphological traits (Fig. 3.5C; on average 92%), except for leaf width and leaf thickness. The variation in most physiological traits (Fig. 3.5A, on average 104%) is larger or equal amongst *Hordeum* spontaneum populations than amongst *Hordeum* species, except for RGR, photosynthesis per unit leaf area. The variation in chemical traits (Fig. 3.5D, on average 137%) was for all traits larger amongst *H. spontaneum* populations than amongst *Hordeum* species.

	Perennial	Annual	P
RGR (mg g^{-1} d^{-1})	222	231	n.s
ULR (g m^{-2} d^{-1})	14.9	16.6	**
SLA (m^2kg^{-1})	32.6	31.7	n.s.
LMF (g g^{-1})	0.48	0.45	*
SMF (g g^{-1})	0.24	0.24	n.s.
RMF (g g^{-1})	0.29	0.31	n.s.
Seed mass (mg)	2.6	16.6	*
Dry mass (g)	0.44	2.06	*
WCleaf (g g^{-1})	5.1	5.5	n.s.
WCstem (g g^{-1})	6.6	8.1	*
WCroot (g g^{-1})	11.6	11.7	n.s.

Table 3.4: Summary of the mean differences between perennial and annual life-forms of fifteen *Hordeum* species (* = $0.05 < P < 0.01$; ** = $P < 0.01$).

The variation width of both the *H. spontaneum* populations and *Hordeum* species differs amongst the four groups of traits (Fig. 3.5), e.g. the variation width of morphological traits seems much larger than in any of the other three groups of traits. Another remarkable observation is that amongst *H. spontaneum* populations the water content of the leaf and amount of nitrate and minerals in the leaf is much higher than amongst *Hordeum* species.

Discussion

Variation in RGR and growth related traits

The RGR of the *Hordeum* species differed for 30%. This difference might be considered relatively small, compared to what was found in other studies e.g. 300% (Poorter & Remkes 1990), or 250% (Marañón & Grubb 1993). However, these were studies across genera. Studies within a genus show smaller differences. Villar et al. (1998) found a 60% difference in RGR in 20 *Aegilops* species and stated that this range was fairly large taking into account that all the species were from the same genus and also from the same life form (annuals). However, this last point does not apply to our study, where nine perennials and seven annuals were used. The 30% difference we found is similar to the range found by Chapin et al. (1989), who used four *Hordeum* species and found, under favourable nutrition, also a 30% difference in RGR. An exception in these intergeneric comparisons is the study of Atkin et al. (1996), who found a large range of 230% difference in RGR comparing six alpine and lowland *Poa* species.

There was a positive correlation between RGR and LAR. This is in agreement with many studies (for a review see Poorter & van de Werf (1998)), where usually a

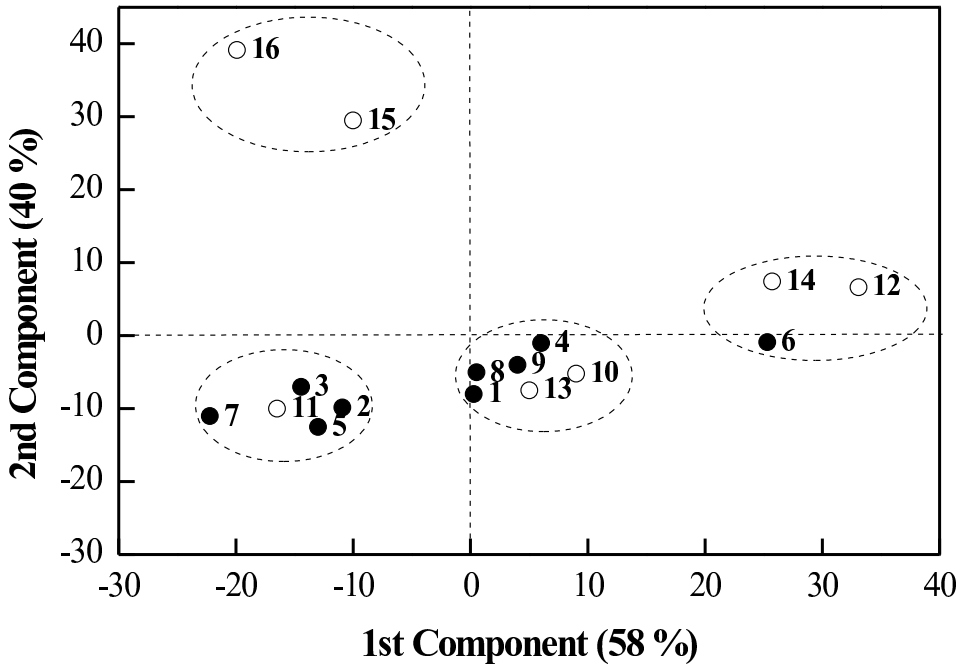

Figure 3.4: Principal Component Analysis of variables, RGR, LAR, ULR, LAR, SLA LMF, SMF, RMF, WCleaf, WCstem, WCroot, Seed. The first component explains 58%, and the second component explains 40% of the total variance. For explanation of species number see Table 3.1. (○ annuals; • perennials)

high correlation between RGR and LAR is found. Most of the variation in LAR was attributed to variation in SLA and to a lesser extent to LMF (Table 3.2). A strong negative relation between ULR and LAR in the *Hordeum* species was observed. Such a relationship between ULR and LAR has been found many times (see, for example, Evans (1972), Poorter & van de Werf (1998) and Villar et al. (1998)). Two explanations are offered for a negative relationship between ULR and LAR by Konings (1989) and Poorter (1989). Firstly, the balance between the amount of roots and the amount of leaf area may affect the water status of the leaves and thereby the rate of photosynthesis. Alternatively, an increase in ULR may require an increase in photosynthetic rate, which can be realised by decreasing the SLA, so that the partitioning of nitrogen over the leaf will be denser. As the negative correlation between ULR and LAR is mainly caused by a negative correlation between ULR and SLA, we expect the second explanation to be the most probable.

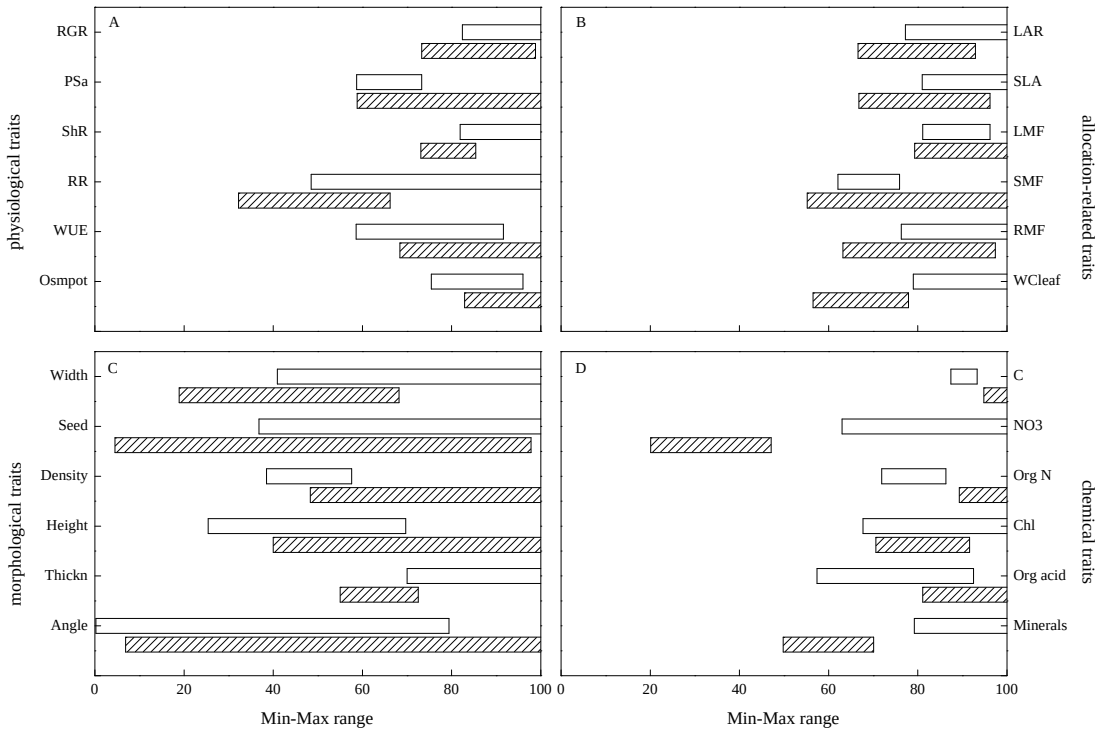

Figure 3.5: Whole range in percentage, of traits measured between 21 populations of *H. spontaneum* (Van Rijn et al. 2000) (open bars) and between *Hordeum* species (hatched bars) of 4 groups of growth traits (A) physiological (B) allocation-related, (C) morphological, (D) chemical traits.

What explains variation in dry mass at day 21?

There was an almost 20-fold difference in biomass at day 21 (circa 28 days after germination) amongst the *Hordeum* species (Fig. 3.3). These differences in biomass were not caused by differences in RGR. There was a positive correlation between seed mass and biomass at day 21. This is in accordance with Chapin et al. (1989), in a study determining plant size in four different *Hordeum* species under favourable nutrition and also with Van Rijn et al. (2000) in a study determining growth characteristics of *H. spontaneum*. In fact there was no correlation between seed mass and RGR (Table 3.3). This agrees with results found by Van Rijn et al. (2000) and Clevering (1999). Meerts & Garnier (1996) found a positive relationship between seed mass and RGR. These are all studies within a species. Usually a negative relationship between seed mass and RGR is found in studies across genera (Shipley & Peters 1990; Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998). Interestingly, when cultivated barley is compared with the wild *Hordeum* species (Fig. 3.3), *H. vulgare* has a low RGR and breeders have probably selected more on seed mass to get a higher yield

than on RGR in this species.

Differences between annuals and perennials

There was no significant difference in RGR between annuals and perennials (Table 3.2). In most studies, annuals have a higher RGR than perennials (Jackson & Roy 1986; Garnier 1992). This is usually explained by the fact that annuals often occur in disturbed or only temporally favourable habitats (Grime 1977; Morishima et al. 1984). Fitness can be increased by growing rapidly in the vegetative phase, but obviously this pattern is not observed in *Hordeum* species. Another way of achieving a high vegetative mass is by allocating a large fraction of energetic and mineral resources to seeds. The annual *Hordeum* species did have a significantly higher vegetative mass and seed mass, hence this is probably the strategy of annual *Hordeum* species to be larger than other species in the field. Only amongst perennial *Hordeum* species there was a positive correlation between biomass allocation to the roots and seed size (data not shown). This is in agreement with Baker (1972), who suggested for the Californian flora that larger seed size might be advantageous in dryer habitats, because of greater allocation to roots in species with larger seeds. However, annual *Hordeum* species did not allocate more biomass to their roots, whereas they did have larger seeds than perennials (Table 3.2).

ULR was larger in annuals than in perennials (Table 3.2). This is in accordance with Garnier (1992), who also studied the growth of congeneric annuals and perennials. ULR is a complex parameter, involving photosynthesis, respiration as well as carbon content (Poorter 1989) In most interspecific comparisons it is strongly correlated with the rate of photosynthesis per unit leaf area (Konings 1989; Poorter & van de Werf 1998). Therefore, Garnier (1992) suggested that the lower unit leaf rates of perennials might be attributed, at least partially, to lower photosynthetic rates. However, in a later study Garnier et al. (1999) observed no difference between these annual and perennial species in photosynthesis per unit leaf area. We did not measure photosynthesis for all fifteen species but we did for six species and also found no significant differences between annuals and perennials ($P > 0.1$).

Principal Component Analysis

The PCA (Fig. 3.4) separates the fifteen *Hordeum* species into four groups of species with RGR as the most important trait on the first axis and seed mass as the most important trait on the second axis. It is hard to explain the separation of the species based on their growth traits considering the broad habitat description (Table 3.1). It shows that *H. spontaneum* from the mediterranean site (Ashqelon) is different from the other

wild species and more similar to the cultivar *H. vulgare*. The other *H. spontaneum* population, from the steppic/marginal mediterranean site (Mehola), has more similarities with the other wild species, in particular *H. chilense* and *H. murinum*. This is particularly interesting, because a cross was made between these two populations of *H. spontaneum*. The offspring of this cross has been used for a QTL-analysis of growth traits, which will be discussed in a later paper.

Atkin et al. (1996) found that alpine *Poa* species had a much lower RGR than *Poa* species from lowland environments. Notably, also for *Hordeum* it seems that in the natural habitat RGR decreases with increasing altitude. *H. comosum* and *H. brevisubulatum* occur at high altitudes in the Andes in South America and at high altitudes in Asia, respectively (Von Bothmer et al. 1995)) and both show low RGR-values (Fig. 3.3; Table 3.1; Appendix 1).

Comparison of variation in *Hordeum* species with variation in *H. spontaneum*

A more detailed growth analysis was performed using six *Hordeum* species originating from five continents and differing in their relative growth rate. The growth traits that were measured on these six species were the same as we measured previously on *H. spontaneum* (Van Rijn et al. 2000). Following this paper we divided the traits into four groups: physiological, allocation-related, morphological and chemical (Fig. 3.5). For almost all traits variation amongst *Hordeum* species was similar to that within *H. spontaneum*. Like in Van Rijn et al. (2000), variation in morphological traits amongst *Hordeum* species is larger than that in the other growth traits.

The expectation was that the variation in *Hordeum* species would be larger than in *H. spontaneum*. However, the variation in *H. spontaneum* is the same as in *Hordeum* species (99%). The variation in chemical traits amongst *H. spontaneum* populations is even larger than amongst *Hordeum* species. Averaged over the six physiological and the six morphological traits the variation is almost the same in *H. spontaneum* and in *Hordeum* species; 104% and 92%, respectively. Also averaged over the six allocation-related traits *H. spontaneum* shows a wide variation: 62% compared to *Hordeum* species. So this means that *H. spontaneum* is a very interesting species for determining variation in growth-related traits.

An interesting observation is that the amount of nitrate and minerals in *H. spontaneum* populations is much higher than in *Hordeum* species (Fig. 3.5). This coincides with a higher water content in the leaves of the *H. spontaneum* populations Therefore it is likely that the higher concentration of minerals is used to maintain a sufficiently negative osmotic potential, as was earlier found in *Lactuca sativa* (Blom-Zandstra et al. 1988).

Conclusions

Variation in RGR was relatively small in *Hordeum* species, but this is mainly due to a strong negative correlation between ULR and SLA. Seed mass is an important trait determining differences in biomass in *Hordeum* species. Annual and perennial *Hordeum* species do not differ in RGR, but annual *Hordeum* species do have a higher seed mass and ULR. As expected, the variation amongst *Hordeum* species is larger than within *H. spontaneum*, and *Hordeum* species differ more in morphology than in physiology of growth traits. The variation in *H. spontaneum* covers for most traits the whole range of variation in *Hordeum* species and is therefore suitable for ecophysiological growth-related studies.

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Appendix 1

Species	Seed mass (mg)	RGR (mg $g^{-1}d^{-1}$)	ULR (g $m^{-2}d^{-1}$)	SLA (m^2 kg^{-1})	LMF (g g^{-1})	SMF (g g^{-1})	RMF (g g^{-1})
H. bogdanii	1.87	225 ± 6.1	13.2 ± 0.54	36.9 ± 0.91	0.47 ± 0.00	0.29 ± 0.00	0.24 ± 0.00
H. brachyantherum	1.65	214 ± 9.1	14.0 ± 0.75	32.2 ± 0.98	0.49 ± 0.00	0.25 ± 0.00	0.26 ± 0.00
H. brevisubulatum	2.95	210 ± 12.8	14.5 ± 0.97	31.9 ± 0.85	0.47 ± 0.00	0.26 ± 0.01	0.27 ± 0.01
H. bulbosum	4.37	232 ± 11.6	15.7 ± 0.90	31.6 ± 0.41	0.47 ± 0.00	0.16 ± 0.00	0.37 ± 0.01
H. capense	2.17	209 ± 8.6	13.7 ± 1.07	32.9 ± 1.66	0.48 ± 0.00	0.21 ± 0.00	0.30 ± 0.01
H. chilense	2.30	251 ± 5.5	15.9 ± 0.76	32.8 ± 1.17	0.50 ± 0.00	0.25 ± 0.00	0.25 ± 0.00
H. comosum	2.48	203 ± 12.1	17.2 ± 1.72	25.9 ± 1.14	0.48 ± 0.01	0.23 ± 0.00	0.29 ± 0.01
H. cordobense	3.35	226 ± 10.0	14.6 ± 0.87	37.1 ± 0.97	0.43 ± 0.00	0.24 ± 0.00	0.33 ± 0.00
H. flexuosum	2.62	230 ± 8.3	15.2 ± 0.77	31.9 ± 0.58	0.50 ± 0.00	0.25 ± 0.00	0.25 ± 0.00
H. intercedens	1.93	233 ± 6.0	15.1 ± 0.51	34.3 ± 1.08	0.47 ± 0.00	0.20 ± 0.00	0.33 ± 0.00
H. marinum	2.49	209 ± 11.8	16.7 ± 1.08	30.6 ± 0.78	0.42 ± 0.00	0.27 ± 0.00	0.31 ± 0.00
H. murinum	8.02	260 ± 6.4	17.8 ± 0.45	33.8 ± 0.64	0.45 ± 0.00	0.27 ± 0.00	0.28 ± 0.00
H. pusillum	2.74	230 ± 7.5	17.0 ± 0.90	30.5 ± 0.89	0.46 ± 0.00	0.27 ± 0.00	0.27 ± 0.00
H. spontaneum Mehola	10.21	253 ± 6.5	16.7 ±0.50	32.7 ± 0.46	0.48 ± 0.00	0.22 ± 0.00	0.30 ± 0.00
H. spontaneum Ashqelon	39.59	223 ± 4.3	16.2 ± 0.30	30.9 ± 0.28	0.46 ± 0.00	0.22 ± 0.00	0.32 ± 0.00
H. vulgare	50.99	216 ± 11.5	17.0 ± 0.80	29.4 ± 0.30	0.44 ± 0.00	0.23 ± 0.00	0.33 ± 0.00

List of 15 *Hordeum* species studied and mean values ± SE (n = 8) of different plant parameters: seed mass (air-dry seed mass); RGR (relative growth rate); SLA (specific leaf area); LMF (leaf mass fraction); SMF (stem mass fraction); RMF (root mass fraction). Mean seed mass was calculated from two batches of 15 to 30 seeds each.

Chapter 4

Mapping Quantitative Trait Loci for growth-related traits in *Hordeum spontaneum*

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Abstract

Numerous papers have studied the morphological and physiological factors determining inherent differences in relative growth rate (RGR). In this study we evaluate the genetic basis for RGR and its underlying components (leaf area ratio, unit leaf rate, specific leaf area, leaf mass fraction) with quantitative trait loci (QTL) analysis. A segregating F_3 population of a cross between two *Hordeum spontaneum* C. Koch populations (Ashqelon $\times$ Mehola) was measured for a range of traits related to growth and the C and N economy and morphological traits. Two QTLs for RGR were found, explaining 27% of the total variation. One of these QTLs, on chromosome 1(7H), co-localizes with a QTL for specific leaf area. This suggests a genetic basis for a positive correlation between RGR and SLA that has been reported by many authors. On

chromosome 4 (4H) overlapping QTLs for photosynthetic rate per unit leaf area and stomatal conductance were found, showing that chromosome 4 is probably an important chromosome for photosynthesis- related traits. No QTLs were mapped for unit leaf rate, but the location with the highest LOD score for unit leaf rate was coinciding with the QTL for photosynthetic rate per area. QTLs for water-use efficiency and photosynthetic nitrogen-use efficiency were located on chromosome 1 and 5 respectively. Morphological traits such as leaf length, leaf width and plant height are probably regulated by common factors on chromosome 2 and 6. Furthermore one QTL for seed mass and one for leaf angle were located on chromosome 2 and 4 respectively.

Introduction

Plant species differ considerably in the relative growth rate (RGR, net increase in biomass per unit biomass already present per unit of time), they can achieve under optimal conditions. This is an intriguing phenomenon, especially as plant species that normally occur in fertile habitats often show higher maximum growth rate than plants from nutrient-poor environments (Grime & Hunt 1975; Lambers & Poorter 1992). RGR might also be an important trait for barley breeders, because cultivated barley (*H. vulgare* L.) has a lower RGR than many related wild *Hordeum* species including *H. spontaneum* C. Koch (Chapter 3). RGR in itself may not be a target for natural selection; instead traits that are linked or underlie RGR may have been selected for (Lambers & Poorter 1992; Poorter & Garnier 1999). Differences in RGR can by explained by differences in leaf area ratio (LAR; total leaf area per total unit plant mass) or unit leaf rate (ULR; rate of increase in plant mass per unit leaf area). LAR is the product of the specific leaf area (SLA; total leaf area per unit leaf mass) and the leaf mass fraction (LMF; total leaf mass per unit total plant mass). ULR is a more complex trait that comprises the carbon gain in photosynthesis minus the carbon use in shoot- and root respiration, also taking into account the carbon concentration of the plant's newly formed biomass. In a survey of 111 published comparative growth experiments, SLA was found to be the most important factor explaining inherent differences in RGR (Poorter & van de Werf 1998). None of these reports determined the genetic background of the relationships between growth traits. In this study we aim to locate the genomic regions that control a suite of growth- related, C and N economy related and morphological traits in the progenitor of cultivated barley.

Barley is a model species for genetic and physiological studies (e.g. Koornneef et al. (1997) and shows a wide range of adaptations to various habitats. Cultivated barley seems to be relatively well adapted to stress including drought, where crops such as wheat fail (Whabi & Gregory 1989). Barley is also one of the model crops for molecular genetic studies in monocotyledons, because it is an annual, diploid self-

pollinating species with a relatively short life cycle. Another reason for using barley as a model crop species is that the primitive landraces and the wild progenitor of barley, *H. spontaneum*, exhibit a large variation in physiology, morphology and genetics, which might be used to improve cultivated barley (Nevo 1992; Ceccarelli et al. 1995; Forster et al. 2000).

A large genetic variation has been found in wild barley populations, using isozyme polymorphisms, restriction fragment length polymorphism (RFLP)-markers and randomly amplified polymorphic DNA (RAPD)-markers, which showed that many alleles are associated with adaptation to specific environments (Nevo 1992). Recently, *H. spontaneum* has become of interest in studying quantitative traits of crop physiology. There has been much interest in studying quantitative traits of agronomic importance in *H. spontaneum*, such as disease resistance, vegetative biomass and number and mass of spikelets, spikes and stems (Nevo et al. 1984). Physiological traits involved in drought and salt tolerance and N-starvation (Robinson et al. 2000; Ellis et al. 2000; Ivandic et al. 2000) have also been studied. Growth traits in *H. spontaneum* populations from different habitats were studied by Van Rijn et al. (2000).

Physiological and growth-related traits are generally quantitative by nature, they are under polygenic control and sensitive to environmental conditions (Prioul et al. 1997). The introduction of DNA-based molecular markers such as simple sequence repeat (SSR) markers and amplified restriction fragment polymorphism (AFLP) markers and advanced mapping technology like QTL analysis made it possible to relate quantitative traits to the genomic loci and provide data on the relative effects of loci and alleles (Forster et al. 2000). The development of dense molecular marker maps, such as in barley, enabled interpretation of the cause-effect relationship among traits (Lebreton et al. 1995; Simko et al. 1997). QTLs for several physiological traits, such as osmotic adjustment (Teulat et al. 1997), SLA (Yin et al. 1999b), and chlorophyll concentration per unit leaf area (This et al. 2000) have been mapped in barley (for a review see Forster et al. (2000))

QTLs have also been found for physiological traits in other species of agronomic importance. In rice (*Oryza sativa*) stomatal conductance, leaf rolling (Price et al. 1997), seedling vigour traits (Redoña & Mackill 1996) and sodium and potassium uptake in determining salt tolerance (Koyama et al. 2001) were mapped. In maize (*Zea mays*) mostly drought-related traits and loci controlling leaf ABA concentration were found (Lebreton et al. 1995; Quarrie 1996; Tuberosa et al. 1998; Sanguinetti et al. 1999). There has also been an interest in growth traits, such as early vigour in *Glycine max* (Mian et al. 1998), early growth in maize in relation with carbon metabolism (Causse et al. 1995), growth traits and enzyme activity in maize (Prioul et al. 1999) and tree growth and architecture in *Populus* (Wu 1998). In all these studies growth was measured as plant height or leaf length rather than a relative increase in biomass,

or relative growth rate. In this paper we aimed to describe growth using the technique of growth-analysis, in order to determine RGR and its underlying growth components. We intended to determine to what extent these growth-related traits are genetically linked and/or caused by common factors. To answer these questions a cross was made between two *H. spontaneum* populations with contrasting growth traits. From the F_2-population a dense AFLP-marker map was constructed (T.K. Vanhala, pers. comm.). The F_3-population was measured for several growth traits i.e. RGR, SLA, ULR, rate of photosynthesis, shoot and root respiration, concentration of nitrogen and carbon of the leaves and some morphological traits, such as seed mass, leaf width and leaf thickness. In this paper we present the results of a QTL analysis on a range of growth traits, and aim to explain relationships among these traits. We expect the control of a number of attributes to be localised at the same place on the genome.

Material and Methods

Plant material and growth

A population of recombinant inbred lines (RILs) was derived from a cross between two *H. spontaneum* populations, originating from Israel: Ashqelon and Mehola. The Ashqelon parent is a coastal population and the Mehola parent was collected in the Jordan Valley, which is a more steppic, marginal Mediterranean habitat (Nevo et al. 1983). More details on these parents can be found in chapters 2 and 3. Eight F_1 plants were selfed to obtain eight F_2 populations which were selfed to produce 233 F_3 lines (T.K. Vanhala pers. comm.). One hundred and forty F_3 lines were used for the analysis of growth-related traits. There are differences in flowering time between the two parents (B.P. Forster, pers. comm.), but we assumed that, since plants were all measured only 21 days after germination, there were no differences in developmental stage.

Seeds were germinated on moistened filter paper in Petri dishes in a refrigerator at 4°C in the dark. After one-week seedlings were transferred into a container with drainage holes, filled with cleaned white beach sand. The sand was saturated with half strength of the following nutrient solution: 603 μM $Ca(NO_3)_2$, 795 μM KNO_3 , 190 μM KH_2PO_4, 270 μM $MgSO_4$, 0.2 μM $MnSO_4$, 0.9 μM $ZnSO_4$, 20 μM H_3BO_3, 0.3 μM Na_2MoO_4, 40 μM Fe-EDTA, 40 μM $FeSO_4$ and 47 μM SiO_2. The container was placed in a growth room for four days, under the following conditions: 14/10 h day/night, 20°C day/night, irradiance of 450 $\pm$ 25 μmol m^{-2} s^{-1}, relative humidity 70%. Thereafter seedlings were transferred to 33 L tanks containing a full-strength, aerated nutrient solution as mentioned above, which was replaced weekly. The plants were then grown for another 14 days. The pH of the nutrient solution was adjusted at

least four times a week to 5.8 with H_2SO_4. To avoid mutual shading, the number of plants on each container varied between 15 and 7, depending on the size of the plants. Plants were rotated four times a week within the growth room. All plants remained vegetative and none showed dead leaves during the experiment.

Map construction

A genetic linkage map was constructed using 233 F_2 plants of the cross 'Ashkelon $\times$ Mehola'. A set of 44 SSR markers (SCRI; Scottish Crop Research Institute, Dundee, Scotland, UK) and 21 AFLP primer combinations following (Qi et al. 1998): E31M55, E33M54, E33M55, E33M61, E35M48, E35M54, E35M61, E37M32, E37M33, E38M54, E38M55, E38M58, E39M61, E40M38, E41M32, E41M40, E42M32, E42M40, E42M48, E42M51 and E45M55 were used. The AFLP protocol was as described in Vos et al. (1995). The reverse SSR primers and the AFLP E-primers were labelled with either 700 or 800 nm infrared dye (IRD700, IRD800) for detection with Li-Cor automated laser sequencer (Li-Cor Inc., Lincoln, NE, U.S.A.). The PCR conditions for each SSR were as described in the SSR manual from SCRI. The scoring was done by eye with the help of Cross Checker (Buntjer 1999) and linkage map constructed using Joinmap ver. 3.0 (Van Ooijen & Stam 2001). Minimum of LOD score 3.0 was used in determining the groups and Kosambi's mapping function was used for calculating the map distances. The maps of the two parents could not be integrated into one. In figure 4.3a and 4.3b AQ are the linkage groups of the Ashqelon parent and ME are the linkage groups of the Mehola parent.

Experimental design

Measurements of growth-related traits were performed on a sample of 140 RILs. To spread the work-load, plants were grown in 25 batches. Each set comprised of one individual from 28 lines plus the two parental lines as controls. After 5 weeks one individual from each of the 140 lines was measured; after 25 weeks, five individuals from each of the 140 lines were measured. Whole plant fresh mass was measured on all five individuals of each line (after blotting the roots gently with tissue paper) circa 14 days after germination, after which each plant was returned to the nutrient solution. In a preliminary experiment, it was established that the blotting had no effect on the RGR of the plants. All five individuals of each line were used, circa 21 days after germination, to determine fresh and dry mass of leaves, stems and roots, leaf area, leaf width, leaf angle, leaf length, shoot height, root length and the number of leaves and tillers. Three of the five individuals were also used to measure leaf photosynthesis under both ambient and saturating light conditions. The other two individuals were used to measure root respiration and leaf and epidermal thickness.

Measurements

Growth analysis-related traits Total leaf area was determined for all plants using a Li-3100 area meter (Licor, Lincoln, NE, USA). Leaf area, fresh and dry mass of the leaves (leaf blades), stems (leaf sheaths) and roots were determined to calculate water content (fresh mass - dry mass / dry mass) of leaf, stem and root (WC_L , WC_S, WC_R, respectively), leaf area ratio (LAR, leaf area per total plant dry mass), specific leaf area (SLA, leaf area per leaf dry mass), leaf mass fraction (LMF, leaf dry mass per total plant dry mass), stem mass fraction (SMF, stem dry mass per total plant dry mass) and root mass fraction (RMF, root dry mass per total plant dry mass). Dry mass was measured after drying the plant material at 70°C, for 48 hours. ULR was estimated under the assumption that the LAR was constant during the seven days of the growth analysis.

Traits related to C and N economy Gas exchange parameters were determined on an area of approximately 7 cm^2 in the middle part of the youngest fully expanded leaf on the main tiller. Measurements of CO_2 and H_2O release and CO_2 uptake were conducted using an IR gas analyser (LI-6262, LICOR, Lincoln, NE) in the differential mode in an open system (Poot et al. 1996; Atkin et al. 1997). Three leaf cuvettes were connected to a data acquisition system (Keithley 575, Cleveland, OH) and measured simultaneously. Air in each cuvette was mixed with a fan. Different light intensities were obtained by placing small-mesh wire netting filters in front of slide projector lamps mounted above each cuvette. Leaf temperature was measured using two thermocouples pressed against the abaxial side of the leaves. After acclimation for 30-45 minutes, CO_2 and H_2O exchange was measured. The conditions in the cuvet were similar to those in the growth room, i.e. 35 Pa CO_2, leaf temperature: 20° C and a PPFD of 450 μmol m^{-2} s^{-1}. Thereafter CO_2 and H_2O exchange were determined at a PPFD of 1500 μmol m^{-2} s^{-1} to assess photosynthesis at light saturation. Finally, plants were placed in the dark for 20 minutes and dark respiration was measured.

Root respiration was determined on detached roots as the decrease of O_2 concentration in an airtight cuvette containing a nutrient solution, which was air-saturated at the start of the experiments. The O_2 concentration was measured using a Clark-type electrode (Yellow Springs Instruments, OH, USA) (Lambers et al. 1993).

To determine the concentration of C, total N, NO_3^- and organic N, the three youngest fully expanded leaves of each line used in photosynthesis measurements, were combined to one sample, which was determined in duplicate. The C- and N-concentration of the samples were quantified with an elemental analyser (Carlo Erba 1110, Italy). Nitrate was determined according to Cataldo et al. (1975). Nitrate concentration was then subtracted from total N to determine the organic N-concentration.

Morphological traits Mass of each air-dried seed (coated caryopsis, without awn or spikelet stalk) was separately determined with a Sartorius R160P (Göttingen, Germany) balance. These seeds contained 6% water on average.

Leaf thickness and epidermal thickness were determined microscopically in the middle of the youngest fully expanded leaf at 5 points: on the main vein, on the fourth vein at each side of the main vein and between the fourth and the fifth vein at both sides of the main vein. Average leaf and epidermal thickness were calculated using these five data points. Leaf width was measured as the average of five points in the middle of the youngest fully expanded leaf. The youngest fully expanded leaf was also used to determine leaf angle. This was done by determining the angle between the horizontal plane and the middle part of the leaf. That is, leaves with a vertical orientation had an angle of 90°. Leaf length was determined on the youngest fully expanded leaf as the distance between the ligule and the top of the leaf blade. Shoot height was determined as the distance between the base and the highest point of the shoot. Root length was determined as the distance between the base and the lowest point of the root.

Statistical analysis Differences between the 140 F_3 lines were calculated with the one-way ANOVA- procedure in SPSS for Windows (release 8.0; SPSS Inc., Chicago, IL, USA). In the QTL analysis plant values of the F_3 lines were regressed on the marker genotypes of the parental F_2 plants.

A computer software package, MapQTL version 4.0 (Van Ooijen & Maliepaard 1996) was used for interval mapping and restricted MQM-mapping (Jansen 1993; Jansen & Stam 1994). In the regions of the sub-significant QTLs, the markers with the highest LOD values were taken as co-factors. When LOD values for some markers in other regions became significant, they were gradually added as cofactors, until the LOD profile stabilised. LOD values of 2.7 were calculated (according to Van Ooijen (1999)) as significant threshold values for declaring a QTL significant at the 95% confidence level. The additive effect and the percentage of the total phenotypic variation explained by each putative QTL were also estimated using the MapQTL software.

Results

Variation in phenotypic data

Table 4.1 shows all the traits that were measured or calculated, categorised into three groups: growth-analysis, C and N economy, and morphological traits. Almost all traits showed significant line differences, except for shoot and root respiration, unit leaf rate, water-use efficiency (ambient and saturated light conditions) and leaf thickness (Table 4.1: ANOVA). Figs 4.1 and 4.2 show the variation in the F_3 population between lines

for mean values of growth analysis-related (Fig. 4.1A-E) and C and N economy-related traits (Fig. 4.1F-L), and morphological traits (Fig. 4.2A-I). The range of the mean values of all traits extends beyond that of the parents (except for seed mass and leaf width), exhibiting transgressive segregation.

Relationships among growth-related traits

There were strong interrelations among growth-related traits (Table 4.2). The measured traits were divided into three groups. The first group are traits that are positively correlated with each other and includes the RGR and allocation traits related to leaf area (LAR and SLA) and water content of the organs (WCleaf, WCstem, WCroot), root respiration and most morphological traits (height, leaf length, angle, width and thickness). The variables of the second group of traits are negatively correlated with those of the first one and correlate positively with each other. They include net dry mass and carbon gain on an area basis (ULR, PS_A), shoot respiration, stomatal conductance and organic nitrogen concentration. A third group of traits containing the leaf density, biomass allocation (LMF, SMF, and RMF), carbon content, water-use efficiency, photosynthetic nitrogen-use efficiency and some morphological traits (seed mass, root length and epidermal thickness) show positive or negative correlations with the other two groups.

QTL mapping

Quantitative trait loci mapping results are presented in Tables 4.3 and 4.4. Significant QTLs are located on chromosomes 1 (7H), 2 (2H), 4 (4H), 5 (1H) and 6 (6H) (Fig. 4.3. The range of variance explained by each QTL ranged from 11% to 29%. In most physiological literature a threshold of 2.0 of the LOD-score is taken as the significance of a QTL. A more conservative threshold value of the LOD -score was calculated in this study, following (Van Ooijen 1999) and was 2.7. QTLs above this threshold are shown in Table 4.3 and Fig. 4.3. A number of QTLs below this threshold are presented in Table 4.4. Most of these sub-significant QTLs have LOD values between 2.0 and 2.7 ($P < 0.19$), but for some important growth-related traits the highest peak LOD value is shown. More than one QTL was found for a number of traits and we tested these for epistatic effects. To test this, only the QTLs on the AQ linkage group were tested, because the QTLs on the ME linkage groups might be the same QTLs as on the AQ linkage group. For RGR, LMF and leaf width a two-factor analysis of variance was applied (two QTLs for each trait) to test whether the QTL effects are additive or whether significant interactions occur between them. For leaf length a three-factor analysis of variance (three QTLs) was applied. No significant interactions among QTLs were detected for any of these traits (data not shown).

Abbrevation	Trait	Unit	Significance in ANOVA	mean Ashqelon	mean Mehola
Growth-analysis					
RGR	relative growth rate	mg g^{-1} day^{-1}	***	252	268
LAR	leaf area ratio	m^2 kg^{-1}	***	18.4	19.3
SLA	specific leaf area	m^2 kg^{-1}	**	38.8	39.5
SLAy	specific leaf area of youngest full-grown leaf	m^2 kg^{-1}	***	33.2	36.3
LMF	leaf mass fraction	g g^{-1}	***	0.48	0.49
SMF	stem mass fraction	g g^{-1}	***	0.22	0.21
RMF	root mass fraction	g g^{-1}	***	0.31	0.30
C and N economy					
ULR	unit leaf rate	g m^{-2} day^{-1}	n.s.	13.7	14.0
PS_A	photosynthesis per unit leaf area	μmol CO_2 m^{-2} s^{-1}	***	16.3	16.7
PS_M	photosynthesis per unit leaf mass	μmol CO_2 g^{-1} s^{-1}	*	545	602
SR	shoot respiration	nmol g^{-1} s^{-1}	n.s.	1.35	1.28
RR	root respiration	nmol g^{-1} s^{-1}	n.s.	58.2	64.5
% Resp	percentage respiration	-	-	24.5	23.6
g_s	stomatal conductance	mmol m^{-2} s^{-1}	*	289	335
WUE	water-use efficiency	mg g^{-1}	n.s.	7.6	7.2
PNUE	photosynthetic nitrogen-use efficiency	μmol CO_2 (mol N)-1 s^{-1}	***	143	157
N_A	nitrogen per unit leaf area	mg m^{-2}	***	2.2	2.0
C	carbon content	mg g^{-1}	***	389	400
NO_3	nitrate	mg g^{-1}	***	84.5	86.8
Org. NA	organic nitrogen per unit leaf area	mmol m^{-2}	***	114	106
Morphological					
WCleaf	water content of leaf	g g^{-1}	***	7.0	7.1
WCstem	water content of stem	g g^{-1}	***	10.2	9.6
WCroot	water content of root	g g^{-1}	***	13.3	12.4
Height	plant height	cm	***	16.9	8.5
Angle	leaf angle	$^\circ$	***	15.0	8.7
Length	leaf length	cm	***	28.5	22.2
Width	leaf width	mm	***	12.3	8.2
Thickness	leaf thickness	μm	n.s.	337	327
Epi Th.	epidermal thickness	μm	*	75.4	70.7
Rootl	root length	cm	***	56.9	52.7
Seed	seed mass	mg	***	50.4	16.4
Density	leaf mass density	g mm^{-3}	***	101	90
# till	number of tillers	-	***	6.2	7.3
# leaf	number of leaves	-	***	18.8	19.4

Table 4.1: Abbrevations of all the traits measured, units, the significance in one-way ANOVA of 140 lines (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = not significant) and the mean values of the Ashqelon and Mehola parent.

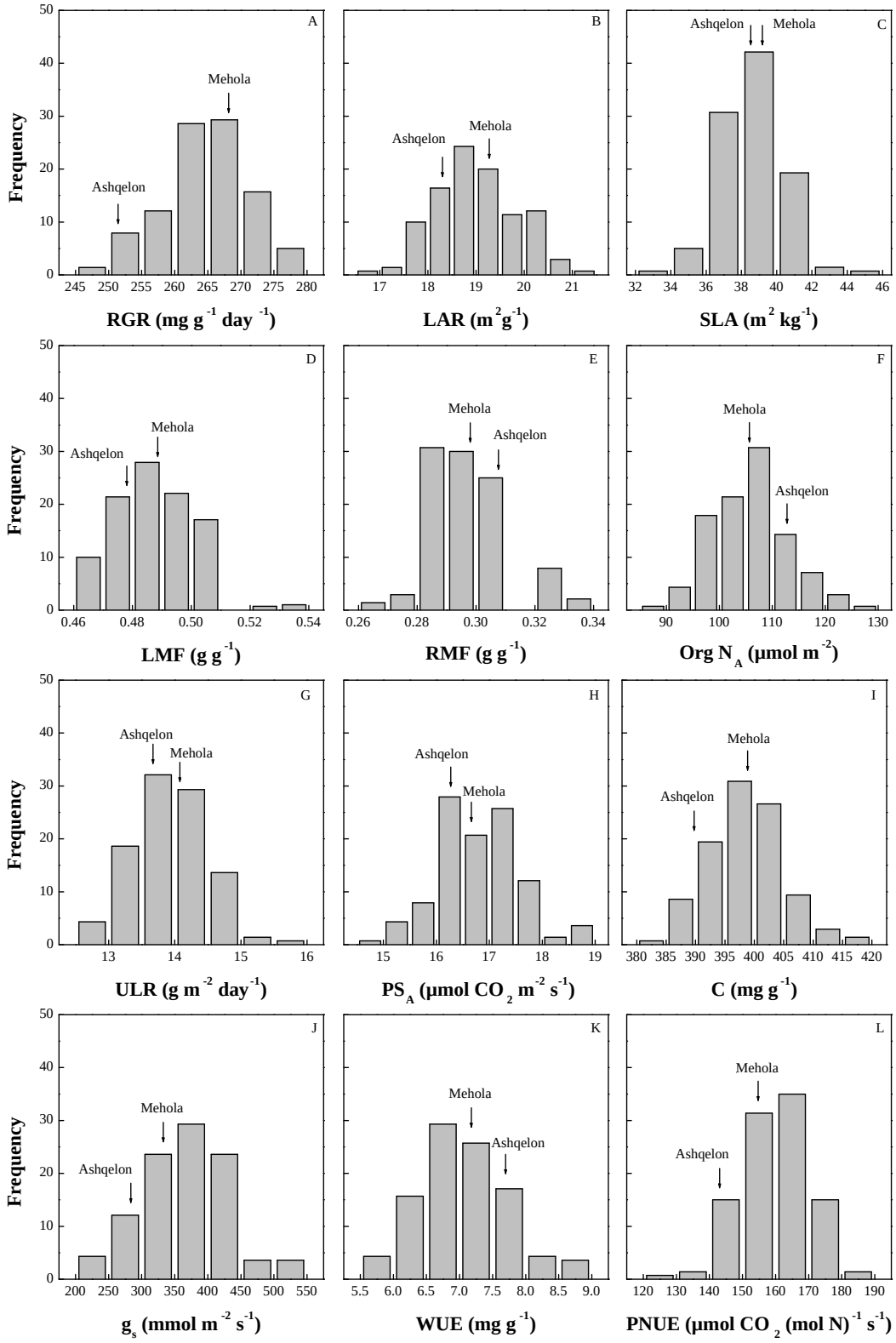

Figure 4.1: Frequency distribution of 140 F_3 lines (A) RGR, (B) LAR, (C) SLA, (D) LMF, (E) RMF, (F) Org N_A, (G) ULR, (H) PS_A, (I) C, (J) g_s, (K) WUE, (L) PNUE.

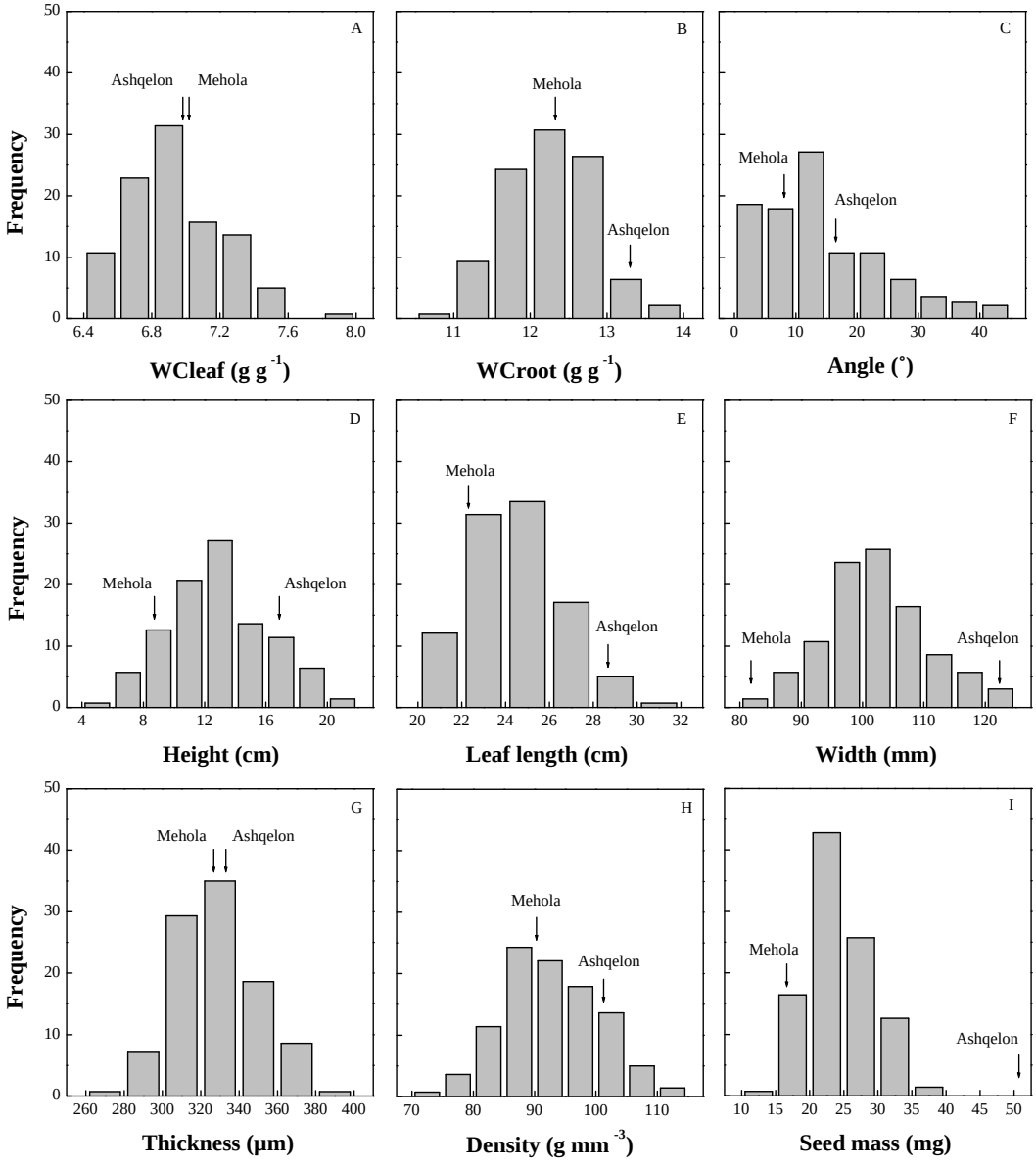

Figure 4.2: Frequency distribution of 140 F_3 lines (A) WCleaf, (B) WCroot, (C) Angle, (D) plant height, (E) leaf length, (F) leaf width, (G) apparent leaf thickness (H) leaf mass density, (I) seed mass

	RGR	LAR	SLA	WCleaf	WCstem	WCroot	RR	Heigth	Length	Angle	Width	Thickness	ULR	PS_A	g_s	SR	Org N_A	Density	LMF	SMF	RMF	Seed	Rootl	PS_M	C	PNUE	WUE
RGR		+	+	+	+	+	+		+		+			-	-		-		+		-	-					
LAR	*		+	+	+	+	+	+	+				-	-	-	-	-	-	+	-	-	-		+			
SLA	*	*		+	+	+		+	+				-	-	-	-	-	-	-		+	-		+			
WCleaf	*	*	*		+	+	+					+	-	-	-	-	-	-	-		+						
WCstem	●	○	*	*		+		+		+	+			-	-	-	-		-		+						
WCroot	○	●	*	*	*											-			-		+	+			-	-	
RR	●	●		○											-				+	+		-	-				
Height		●	○		*				+	+	+	+	-	-			-			+		+					
Length	○	○	●					*		+	+	+	-	-			-				-	+	+				
Angle					*			*	○		+			-						+							
Width	●				●			*	*	○		+		-				+				+	+				
Thickn				●				●	●		○							+					+	-			
ULR		*	*	*				●	●					+	+	+	+	+	-	+				-			
PS_A	*	*	*	*	*			●	●	●	●		*		+	+	+				+	+		+			
g_s	○	*	*	*	*		●						*	*		+	+				+			+			
SR		●	*	*	*	*							●	*	○		+	+									
Org N_A	*	*	*	*	*			●	*				*	*	*	○		+				+					
Density		*	*	●							○	*	●			●	*		+		-	+	+	-			
LMF	○	*	●	●	*	*	*						○					○		-	-	-		-			
SMF		●					●	*		○			●						*		-	+					
RMF	○	●	●	●	*	*			○					●	●			○	*	*				-			
Seed	*	*	*			○	○	●	●		*			●			*	*	●	●			+		-		
Rootl							●		*		*	○						●				●					
PSM			*									●	●	*	*			*	*		*						
C						○																				+	-
PNUE						●																●			*		-
WUE																									*	●	

Table 4.2: Correlation table of growth traits in *H. spontaneum*. The upper part of the table shows the significant positive or negative correlation. In the lower part of the table the ● denotes significance at $P < 0.05$, ○ denotes significance at $P < 0.01$ and ∗ denotes the strongest correlations with a $P < 0.001$.

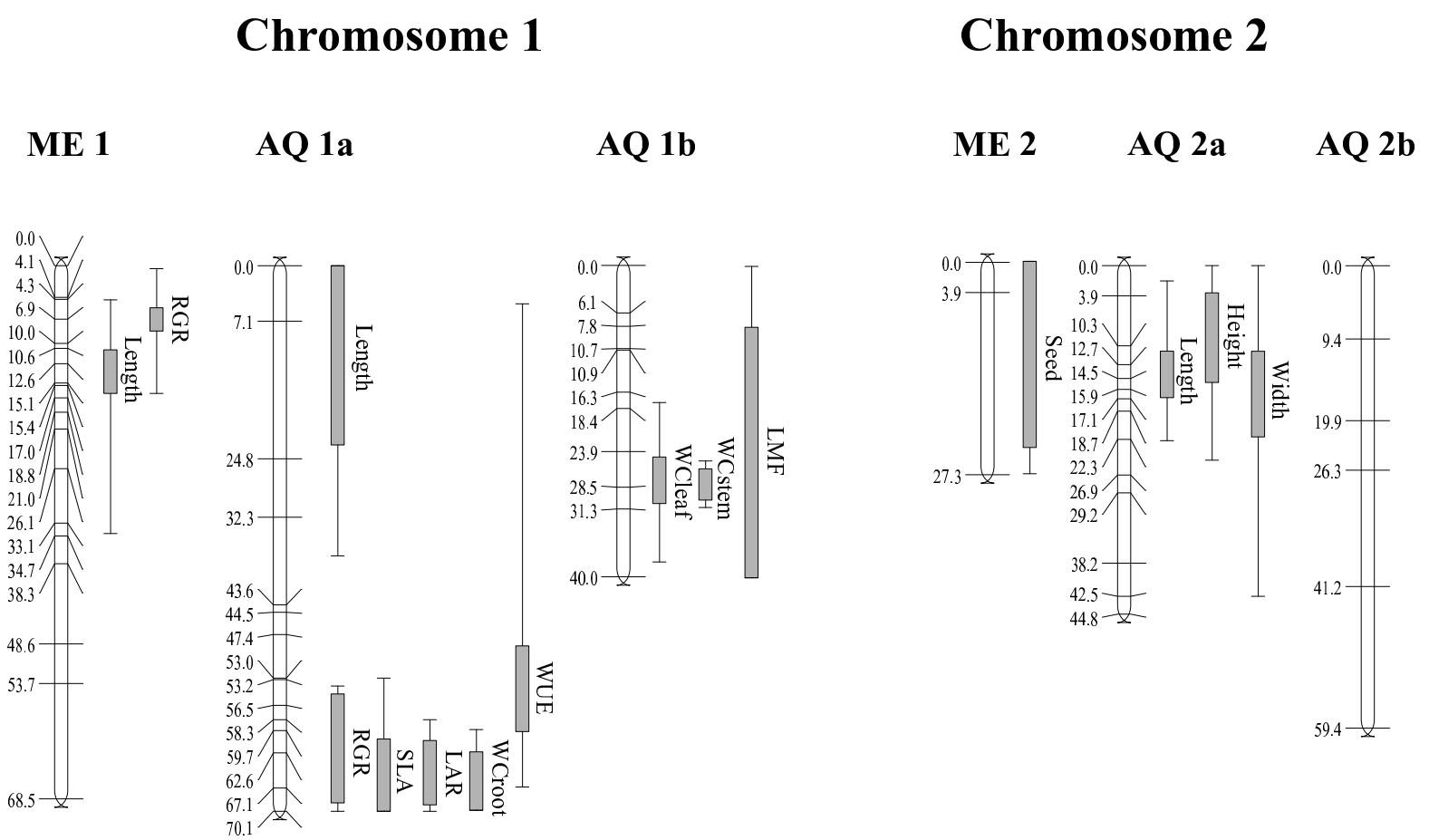

Figure 4.3.a: AFLP marker linkage map of *H. spontaneum chromosomes* (A) 1 and 2 (LG ME1, AQ1a AQ1b, ME2, AQ2a, AQ2b) (B) 4, 5 and 6 (LG ME4, AQ4a, AQ4b, ME5, AQ5, ME6, AQ6) The positions of all QTLs above the LOD threshold of 2.7 are shown. Map positions are given in cM using the Kosambi function. Bars indicate the QTL locations with a two LOD support confidence interval.

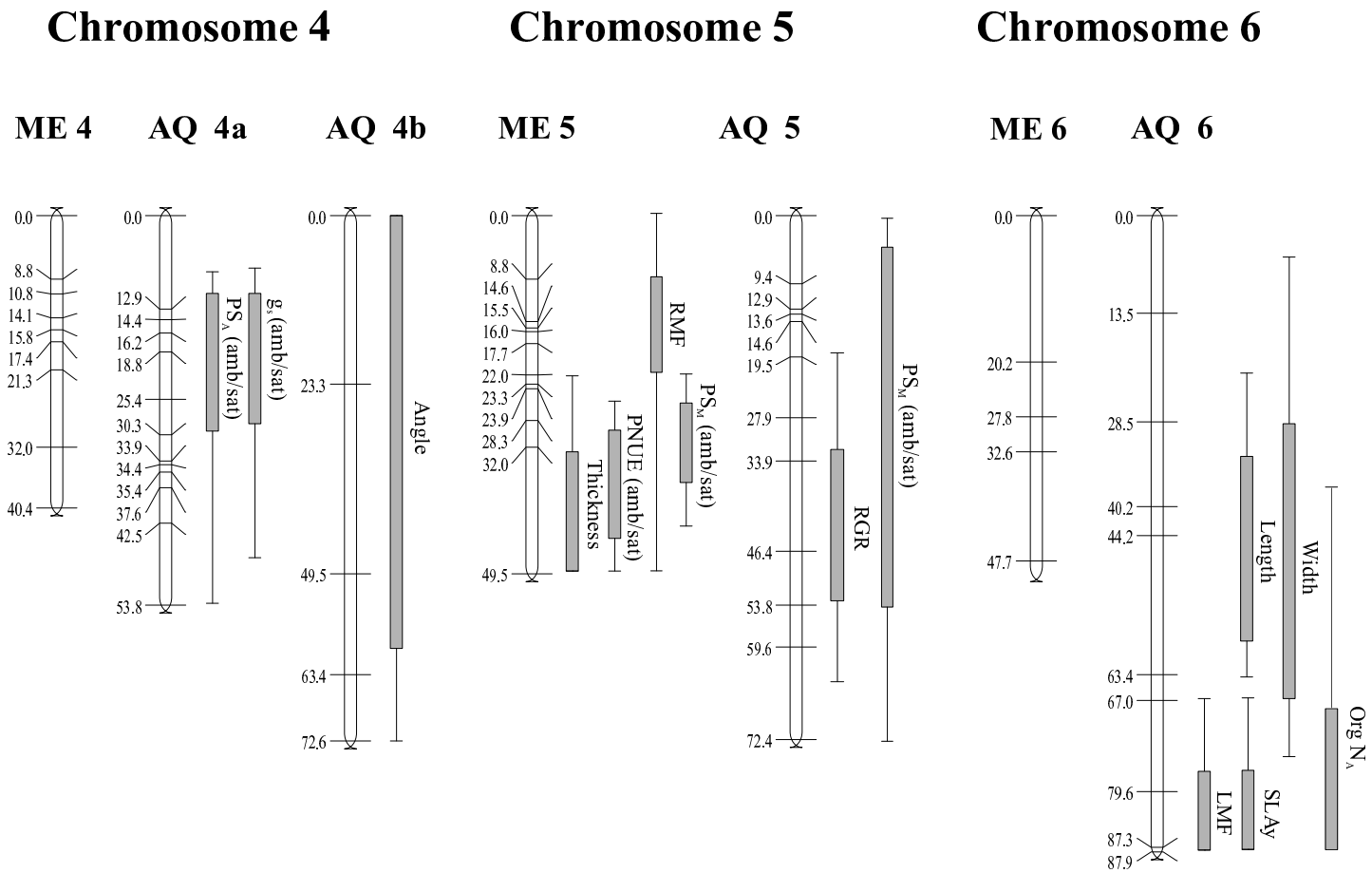

Figure 4.3.b: AFLP marker linkage map of *H. spontaneum chromosomes* (A) 1 and 2 (LG ME1, AQ1a AQ1b, ME2, AQ2a, AQ2b) (B) 4, 5 and 6 (LG ME4, AQ4a, AQ4b, ME5, AQ5, ME6, AQ6) The positions of all QTLs above the LOD threshold of 2.7 are shown. Map positions are given in cM using the Kosambi function. Bars indicate the QTL locations with a two LOD support confidence interval.

QTLs	co-factor marker	chromo-some	Interval length[a] (cM)	QTL Position (cM)	Additive effect[b] (%)	Peak LOD score	% variance explained[c]
Growth-analysis							
RGR	MeE42M51-280	ME1	16.0	6.90	1.2	2.8	12
RGR	AqE33M54-568	AQ1a	16.1	62.6	-1.4	3.6	16
RGR	AqE38M58-142	AQ5	45.5	46.4	-1.2	2.8	11
LAR	AqE35M48-450	AQ1a	11.8	67.1	-2.7	3.5	17
SLA	AqE35M48-450A	AQ1a	17.1	67.1	-2.5	2.8	12
SLAy	AqE42M48-130	AQ6	20.9	87.9	3.3	2.8	25
LMF	AqE35M48-170	AQ1b	40.0	18.4	1.3	2.9	13
LMF	AqE42M48-130	AQ6	20.9	87.9	-2.0	3.2	25
RMF	MeE38M55-510	ME5	49.5	14.6	2.7	3.0	17
C and N economy							
PSA (amb)	AqE45M55-206	AQ4a	45.8	14.4	3.2	2.8	20
PSA (sat)	AqE45M55-206	AQ4a	45.8	14.4	4.9	2.9	23
PSM (amb)	MeE38M58-160	ME5	17.7	32.0	3.7	5.3	23
PSM (sat)	MeE33M61-340	ME5	21.0	28.3	4.7	5.1	26
PSM (amb)	AqE35M61-285	AQ5	72.4	9.4	-3.0	3.3	21
PSM (sat)	AqE41M40-445	AQ5	72.4	33.9	-3.1	2.9	12
g_s (amb)	AqE45M55-206	AQ4a	40.0	14.4	12.0	2.9	22
g_s (sat)	AqE45M55-206	AQ4a	39.5	14.4	12.2	3.5	24
WUE	AqE35M54-405	AQ1a	62.1	53.0	4.5	2.8	11
PNUE (amb)	MeE38M58-160	ME5	23.5	32.0	3.5	2.7	13
PNUE (sat)	MeE38M58-160	ME5	24.5	32.0	4.2	3.6	17
Org N_A	AqE35M48-246	AQ6	50.4	87.3	-5.0	2.9	23
Morphological							
WCleaf	AqE41M40-192	AQ1b	20.5	28.5	-2.4	3.5	16
WCstem	AqE41M40-192	AQ1b	6.0	28.5	-3.1	5.4	25
WCroot	AqE33M54-205	AQ1a	10.4	70.1	-3.1	3.7	20
Height	AqE33M54-518	AQ2a	25.0	12.7	13.9	2.9	15
Angle	AqE42M40-267	AQ4b	72.6	23.3	38.4	3.1	14
Length	MeE39M61-120	ME1	30.0	15.1	5.2	4.7	19
Length	AqE42M40-332	AQ1a	37.3	7.10	-5.7	3.5	21
Length	AqE33M54-518	AQ2a	20.5	12.7	4.9	4.1	17
Length	AqE35M61-106	AQ6	42.0	44.2	4.1	3.5	14
Width	AqE33M54-518	AQ2a	42.5	12.7	4.1	3.0	12
Width	AqE37M32-235F	AQ6	69.0	40.2	4.2	3.3	14
Thickness	MeE41M40-304E	ME5	27.0	49.5	-3.8	2.8	15
Seed mass	MeE33M61-182	ME2	27.3	0.00	-13.3	2.7	29

[a] The support interval was estimated at a LOD fall off -2.00

[b] On Ashqelon map (mean of the Ashqelon allele genotype - mean of the Mehola allele genotype)/2
On Mehola map (mean of the Mehola genotype - mean of the Ashqelon allele genotype)/2

[c] Percent explained phenotypic variance

Table 4.3: Quantitative trait loci for growth traits in a *H. spontaneum* F_3 population.

suggestive QTLs	co-factor marker	chrome-some	suggestive QTL Position (cM)	Additive effect[b] (%)	Peak LOD score	% variance explained[c]
Growth-analysis						
RGR	MeE40 M38-323	ME3	25.0	1.2	2.3	10
LAR	MeE42M51-280	ME1	6.9	-2.2	2.4	13
LAR	MeE45M55-86B	ME3	27.3	-2.7	2.5	18
LAR	AqE45M55-206	AQ4a	14.4	-2.6	2.0	12
SLA	AqE41M40-192	AQ1b	28.5	-1.9	1.9	9
SLA	AqE42M40-452	AQ3	26.1	2.0	1.8	9
ySLA	AqE38 M55-433E	AQ5	53.8	-2.0	2.4	14
LMF	MeE38M54-238	ME5	0.0	1.4	2.1	12
LMF	MeE33M54-317G	ME7	0.0	1.3	1.9	10
LMF	AqE35M48-391	AQ4a	42.5	-1.5	2.5	13
RMF	AqE35M48-91B	AQ1b	6.1	-1.9	1.9	10
C and N economy						
ULR	AqE35M54-318	AQ4a	33.9	1.6	1.3	7
PS_A (amb)	AqE37M33-428	AQ3	38.9	-2.5	2.0	11
PS_A (sat)	AqE33M54-128C	AQ3	82.4	-3.8	2.6	18
PS_M (amb)	AqE42M48-130	AQ6	87.9	3.1	2.0	19
SR	AqE42M32-231A	AQ1a	0.0	4.7	2.5	16
%Resp	AqE33M61-123	AQ1a	32.3	3.2	2.3	12
%Resp	AqE37M33-428	AQ3	38.9	3.7	2.1	12
g_s (sat)	AqE33M54-128C	AQ3	82.4	-8.0	2.0	13
WUE (amb)	MeE31M55-262	ME1	4.1	4.3	2.3	10
WUE (amb)	AqE37M33-189	AQ4a	53.8	-4.8	2.0	12
WUE (sat)	AqE35M54-405	AQ1a	53.0	3.2	1.8	5
PNUE	AqE45M55-417	AQ6	67.0	3.4	1.8	11
C	AqE33M55-178	AQ5	12.9	0.8	1.9	11
Org N_A	AqE45M55-206	AQ4a	14.4	3.5	1.8	11
Org N_A	AqE45M55-407	AQ3	64.4	-3.3	2.4	17
morphological						
WCleaf	MeE42M48-98	ME1	12.6	-2.0	2.3	11
WCstem	MeE33M54-317G	ME7	0.0	-2.1	2.3	11
WCstem	AqE35M48-91B	AQ2b	0.0	1.6	1.9	5
WCroot	MeE42M51-280	ME1	6.9	-2.2	2.4	11
Height	AqE39M61-505F	AQ6	28.5	12.0	2.6	11
Height	AqE42M40-267	AQ4b	23.3	11.2	1.9	9
Length	MeE39M61-360G	ME7	62.4	4.2	2.1	13
Length	AqE35M48-169	AQ1b	0.0	3.7	2.0	7
Width	MeE33M61-200	ME1	26.1	-4.0	2.0	11
Width	AqE35M48-91B	AQ2b	0.0	3.7	2.3	10
Epi Th.	MeE42M48-98	AQ1	12.6	-3.2	1.8	8
Rootl	AqE45M55-465	AQ2a	22.3	5.7	2.4	12
Seed	AqE41M40-445	AQ5	33.9	7.0	1.7	7
Dens	AqE39M61-530	AQ2b	19.9	-3.2	2.0	10
# till	AqE33M55-329	AQ4b	49.5	-10.5	2.3	26

[b] On Ashqelon map (mean of the Ashqelon allele genotype - mean of the Mehola allele genotype)/2
On Mehola map (mean of the Mehola genotype - mean of the Ashqelon allele genotype)/2

[c] Percent explained phenotypic variance

Table 4.4: Sub-significant quantitative trait loci for growth traits in a *H. spontaneum* F_3 population.

QTLs for growth analysis traits

On linkage group AQ1A and AQ5, on chromosome 1 (7H) and 5 (1H), respectively, a QTL was found for RGR. Since there are no interactions between them, together they explained (16% +11%) 27% of the total variance. The QTL on the ME1 linkage group is probably the same as the one on the AQ1a linkage group. The additive effects were -1.4% and -1.2% and contributed by the Ashqelon parent. On chromosome 1 (7H) some other QTLs were overlapping with the QTL for RGR. LAR, SLA and WCroot explained 17%, 12% and 20%, respectively, of the total variance. The additive effects of these traits were negative, similar to the ones for RGR, and were contributed by the Ashqelon parent. On chromosome 5 (1H) a QTL, explaining 21% of the total variation in PS_M, coincided with the QTL for RGR. The additive effect of this QTL was also negative; it was caused by the Ashqelon allele. Seed mass and SLA of the youngest fully expanded leaf (SLAy) had sub-significant QTLs on chromosome 5 (1H) with LOD -scores of 1.7 and 2.4, respectively, at positions that would probably overlap with the QTL for RGR (Table 4.4). The additive effects were positive for seed mass and negative for SLAy. Two QTLs were found for leaf mass fraction on chromosome 1 (7H) and 6 (6H), explaining 38% (13% + 25%) of the total variance. For RMF one QTL was found, which explained 17% of the total variance. Two QTLs for WCleaf and WCstem overlapped with the QTL for LMF on chromosome 1 (7H) and explained 16% and 25%, respectively of the total variance.

QTLs for C and N economy traits

QTLs for the rate of photosynthesis per unit leaf area, under ambient as well as saturated light conditions, were found on chromosome 4 (4H). The total variance explained by these QTLs was between 20 and 24%. For all QTLs the Ashqelon allele increased the levels of photosynthesis (3.2% - 4.9%). QTLs for stomatal conductance, under ambient as well as saturated light conditions, were also found on chromosome 4 (4H), co-locating with the QTLs for photosynthesis. The total variance explained by these QTLs was between 22 and 24%, and also the Ashqelon allele increased the levels of stomatal conductance. On chromosome 3 (3H) more sub-significant QTLs for photosynthesis and stomatal conductance were found, but with a negative additive effect. For ULR no significant QTL was found. The highest peak LOD -score (1.3) was found on chromosome 4, probably overlapping with the QTLs found for photosynthesis and stomatal conductance.

A QTL for WUE under ambient conditions was found on chromosome 1 (7H), which overlapped partly with the QTL for RGR. It explained 11% of the total variance in WUE and the Ashqelon allele increased the level for WUE. The QTL for WUE on the Mehola map, under saturated light conditions, was not significant and has been

listed as sub- significant (Table 4.4).

Photosynthetic nitrogen-use efficiency (under ambient and saturated light conditions) was located on chromosome 5 (1H) and explained 13% to 17% of the total variance of PNUE. The additive effects were positive and contributed by the Mehola parent. One QTL for organic nitrogen per unit leaf area was identified on chromosome 6 (6H), explaining 23 % of the total variance. The Ashqelon allele was responsible for a 5% decrease in organic nitrogen per unit leaf area. The highest peak LOD -score for carbon concentration was 1.9; it was positioned on chromosome 5 (1H).

QTLs for morphological traits

Three QTLs for leaf length were identified on chromosomes 1 (7H), 2 (2H) and 6 (6H), which explained 21%, 17% and 14% of the total variance, respectively (total is 52%). On chromosomes 2 (2H) and 6 (6H) two QTLs for leaf width were found, overlapping with the QTLs for leaf length; they explained (12% + 14%) 26% of the total variance. On chromosome 2 (2H) a QTL for plant height was found overlapping with the QTLs for leaf length and leaf width. This QTL explained 15% of the total variance. A QTL for leaf angle was found on chromosome 4 (4H); it explained 14% of the total variance. For all these morphological QTLs, except for the one for leaf length on chromosome 1 (7H), the Ashqelon allele had a positive additive effect on these traits. For plant height and especially for leaf angle these additive effects were relatively high, 14% and 38%, respectively. A QTL for leaf thickness was found on the linkage group of the Mehola parent on chromosome 5 (1H). It explained 15% of the total variance and overlapped with the QTL for PNUE, but with an opposite additive effect. A QTL for seed mass was found on chromosome 2 (2H) explaining 29% of the total variance and with a negative additive effect caused by the Mehola allele.

A sub-significant QTL for leaf mass density was located on linkage group AQ2b, where no other QTLs were found. A sub-significant QTL for root length was located on linkage group AQ2a, probably overlapping with the other morphological QTLs. A sub-significant QTL for the number of tillers was located on linkage group AQ4b overlapping with the QTL for leaf angle. A QTL for WCroot was found on the other linkage group of chromosome 1 (7H) and overlapped with the QTLs for RGR, LAR and SLA. This QTL explained 20% of the total variance.

Discussion

Variation in phenotypic data

Variation in RGR and its underlying components has been studied extensively, but to what extent these traits are genetically linked and/or caused by common factors is un-

known. We therefore tried to locate these traits on the genome using QTL analysis, to reveal the place of control of these traits, and to determine coincidence of some traits on the genome. One of the prerequisites of QTL analysis is that there is a mapping population of plants displaying genetic variability as well as phenotypic variability (Prioul et al. 1997; Stam 1998). This can be achieved by crossing two populations that already differ in the trait of interest. It was shown (Chapter 3) that the two parents (Mehola and Ashqelon), used for the cross, showed large differences for some traits. Also in this study, the parental populations indicated that variation in most traits was sufficiently large for QTL detection. For some traits the parents did not differ significantly, but transgressive segregation occurred, leading to a larger variation in the F_3 population. The exceptions were seed mass and leaf width, where parental variation was greater than the extremes of the F_3 population.

QTLs for RGR and growth analysis traits

Two QTLs for RGR were detected on chromosomes 1 (7H) and 5 (1H). The QTL on chromosome 1 (7H) overlapped with QTLs for SLA and LAR. The additive effects for all three QTLs were in the same direction, and this was confirmed by correlation (Table 4.2), where there was a strong positive correlation between all three traits. The QTL for RGR on chromosome 5 (1H) also overlapped with a sub-significant QTL for SLA of the youngest fully expanded leaf.

LAR can be analysed further as the product of SLA and LMF, which are all simple ratios that can be easily measured. SLA has often been found to be the most important factor explaining inherent differences in RGR (Poorter & Remkes 1990; Garnier 1992; Hunt & Cornelissen 1997; Poorter & van de Werf 1998). This study suggests that there is a genetic basis for this relationship. We expected to find more coincident QTLs for RGR and SLA, but there are no indications for this. RGR is most probably a complex composite physiological trait, which is controlled by several loci on the genome. Problems arise when QTLs only have a small individual contribution and are therefore more difficult to detect (Kearsey & Farquhar 1998b). The QTL analysis may provide few major QTLs with large effects, but may fail to detect many QTLs with small effects. The presence of two QTLs for both RGR and SLA identified on the same location may be due to two closely linked genes or to mechanistic dependency. A denser marker map might help resolve this question, but it is also interesting to search for candidate genes that have been located in identified areas.

One possibility is a gene, controlling both RGR and SLA, which is influenced by hormones, such as gibberellin or abscisic acid. A positive correlation between RGR and the endogenous GA concentration was found for maize by Rood et al. (1990) and for tomato by Nagel et al. (2001). Application of GA increased leaf expansion in

bean (Brock & Cleland 1989) and in barley (Smith et al. 1996) and increased RGR and SLA in *Plantago major* (Dijkstra et al. 1990) and SLA in two *Aegilops* species (Bultynck 2001). Nagel et al. (2001) found that GA-deficient tomato mutants had a decreased RGR and SLA. There are GA-insensitive dwarfing genes mapped in cultivated barley on chromosome 2 (2H) (Börner et al. 1999) and on chromosome 4 (4H) (Ivandic et al. 1999), but to our knowledge not on chromosome 1 (7H). It would in this respect be interesting to identify the genome locations of GA biosynthesis genes in *H. spontaneum.*

There are enzymes that may play a role in determining relative growth rate. In maize physiological and growth QTLs were located in the same regions and a strong correlation between invertase activity and growth (days required for leaf 3 to fully mature) has been found (Causse et al. 1995). A probable explanation would be that in those regions several genes encoding enzyme activity are very tightly linked. As (Bettey et al. 2000) stated, one should not infer too much from coinciding QTLs, because it is very likely that several candidate genes are within the confidence limits of a QTL.

The other component of the LAR is the fraction of biomass allocated to the leaves. Two QTLs for LMF were found on chromosome 1 (7H) and 6 (6H). The QTL on chromosome 1 (7H) overlapped with the QTLs for WCleaf and WCstem. LMF and WCleaf or WCstem had an opposite additive effect, which was confirmed by the negative correlation between these variables (Table 4.2). This means that plants with a high allocation of dry mass to the leaves usually have lower water content in the leaves. On chromosome 6 (6H) a QTL for LMF was found with an opposite additive effect. This QTL overlapped with a QTL for SLAy that had an opposite additive effect; this was confirmed by the correlation analysis. It also overlapped with a QTL for organic nitrogen per unit leaf area, with an additive effect in the same direction, but not supported by the correlation analysis. This means that plants with a high biomass allocation to the leaves - have a low SLAy and a high concentration of organic nitrogen per unit leaf area.

QTLs for C and N economy

A QTL for photosynthetic rate has been mapped in *Helianthus anuus* (Hervé et al. 2001), but in some studies photosynthetic rate is discussed without measuring it. Traits related to photosynthetic rate, such as SLA and transpiration efficiency, have been measured frequently; they are then discussed in relation with photosynthetic rate (Mian et al. 1998; Thumma et al. 2001). QTLs for Rubisco concentration, soluble protein and nitrogen in flag leaves of rice have also been mapped (Ishimaru et al. 2001). In the present study we measured photosynthetic rate and associated traits. A QTL for

PS_A on chromosome 4 (4H) co-localised with a QTL for stomatal conductance. Both QTLs had a positive additive effect in the same direction, which was supported by the correlation analysis. A positive correlation between photosynthetic capacity and stomatal conductance is known for quite some time (Wong et al. 1979; Körner et al. 1979). The diffusion of CO_2 into leaf depends on the stomatal conductance and the difference in CO_2 in and outside the leaf (Von Caemmerer & Farquhar 1981). The intercellular CO_2 concentration was similar amongst the F_3-lines (data not shown), as was also found in a comparison of 24 species differing in relative growth rate (Poorter & Farquhar 1994) as well as in 27 species native to the temperate forest region in Japan (Yoshie 1986). This suggests that the intercellular CO_2 pressure is not important in determining variation in PS_A. Interestingly, no coinciding QTLs were mapped in *Helianthus annuus* for PS_A and g_s (Hervé et al. 2001). A good reason to believe that the coincidence of the QTLs for PS_A and g_s is caused by one gene is the mapping of the Rubisco activase gene (*Rca*) on a *H. spontaneum* map on chromosome 4 (4H) (Becker & Heun 1995). Rubisco activase is an important physiological enzyme because CO_2 assimilation is mediated by Rubisco and Rubisco is activated by Rubisco activase (Farquhar et al. 1982). It is possible that Rubisco activase controls processes like the rate of photosynthesis per unit leaf area and perhaps, indirectly, the stomatal conductance. Another interesting detail is that a QTL for total chlorophyll concentration per unit leaf area was also found on chromosome 4 (4H) in barley (This et al. 2000). This makes chromosome 4 (4H) a very important location for many traits related to photosynthetic traits.

No significant QTL was found for ULR, but the location with highest peak LOD score was found on linkage group AQ4a, probably overlapping with the QTLs for PS_A and g_s. ULR is a complex trait that involves the carbon gain via PS_A, carbon use via respiration and carbon content (Poorter 1989). Photosynthetic rate is the most important trait explaining differences in ULR and is often positively correlated with ULR (Konings 1989; Van der Werf et al. 1998; Evans 1998). A QTL for photosynthetic rate per unit leaf mass was found on chromosome 5 (1H) coinciding with a QTL for RGR. It has often been demonstrated that RGR of young seedlings under controlled conditions is strongly correlated with photosynthetic rate, especially on a mass basis (Poorter et al. 1990; Reich et al. 1992; Walters et al. 1993; Kitajima 1994; Walters & Reich 1996). Photosynthetic rate per unit leaf mass is calculated as the photosynthetic rate per unit leaf area times the SLA. Poorter et al. (1990) stated that this strong correlation between photosynthetic rate per unit leaf mass and RGR is mainly due to differences in SLA. This is confirmed by our data since the sub-significant QTL for SLAy (SLA of the youngest fully expanded leaf and the leaf that was used in photosynthesis measurements) is also located on chromosome 5 (1H).

A QTL for photosynthetic nitrogen-use efficiency (PNUE, carbon gain per unit

leaf nitrogen) was present on chromosome 5 (1H). This QTL overlapped with a QTL for leaf thickness having an opposite additive effect, which was not confirmed in the correlation analysis. There might be a reasonable biological explanation considering that plants with thick leaves seem to have a lower concentration per unit leaf mass of the enzyme Rubisco (Field & Mooney 1986; Evans 1989b). An alternative explanation is that plants with a high SLA often have a high PNUE (Poorter & Evans 1998b), and SLA is often negatively correlated with leaf thickness.

QTLs for morphological traits

All QTLs for water content in leaves stems and roots were located on chromosome 1(7H). This is in accordance with Teulat et al. (1997) who studied barley under water stress and in an irrigated treatment, and found a QTL for relative water content under the irrigated regime on chromosome 1 (7H). Unfortunately, the use of different markers made it impossible to compare the location of the QTLs in the two studies. A sub-significant QTL for SLA was found on the same linkage group of chromosome 1 (7H) as the QTL for water content of the leaf. The correlation analysis also showed a strong positive correlation between the two traits as was also found by Garnier & Laurent (1994a) in a study of 14 congeneric annual and perennial grass species. Garnier & Laurent (1994a) also found that plants with a high SLA and high WCleaf show a higher proportion of mesophyll tissue and lower proportion of sclerenchyma and vascular tissue (which might therefore favour leaf productivity) than plants with a low SLA and low WCleaf, which might favour leaf persistence. In the present study the Mehola parent had a high SLA and high WCleaf. The site of origin of this parent is more xeric than that of Ashqelon, and might therefore favour leaf productivity over leaf persistence so that the plant's life cycle is completed before extreme drought conditions occur. SLA is the reflection of both leaf mass density and leaf thickness (Witkowski & Lamont 1991). Garnier & Laurent (1994a) and Van Arendonk & Poorter (1994) found that in grass species leaf mass density is more important than leaf thickness in explaining differences in SLA (but see Atkin et al. (1996)). Although, we found a strong negative correlation between SLA and leaf mass density in our study, we found no evidence for coinciding QTLs or sub-significant QTLs for SLA and either leaf mass density or leaf thickness.

On chromosome 2 (2H) and 6 (6H) coincident QTLs for leaf length and leaf width were found. A QTL for plant height was also found on chromosome 2 (2H), and chromosome 6 (6H) showed a sub-significant QTL for plant height. These three morphological traits seem to be highly correlated and all had a positive additive effect contributed by the Ashqelon allele. In maize, QTLs for these three morphological traits were found at the same location (Causse et al. 1995). In *H. spontaneum* po-

pulations plants from xeric environments had narrower leaves (Van Rijn et al. 2000). However we have to consider that the environmental data were not directly measured at the site of origin of these populations (Van Rijn et al. 2000). In this study the broader and longer leaves originate from the Ashqelon parent, which grows in a more humid habitat than the Mehola parent. An explanation for this is that plants from drier habitats have smaller leaves to reduce transpiration (Von Willert et al. 1992). This was partly demonstrated in this study by the opposite additive effect of the QTL for WUE and partly overlapping QTL for leaf length on chromosome 1 (7H). Loci for plant height have been mapped in cultivated barley on chromosome 1 (7H), 3 (3H) and 6 (6H) (Hayes et al. 1993).

A sub-significant QTL for seed mass was found to overlap with the QTL for RGR on chromosome 5 (1H). This QTL had an opposite additive effect on seed mass to the one for RGR. This was also supported by the correlation analysis, where a negative correlation between the two traits was observed. In interspecific studies across genera a negative relationship is frequently found between seed mass and RGR (Shipley & Peters 1990; Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998). In intraspecific studies this relationship is not found (Clevering 1999; Van Rijn et al. 2000) (Chapter 2) or a positive relationship is found (Meerts & Garnier 1996). No QTL overlapped with the QTL for seed mass found on chromosome 2 (2H), which explained a large part (29%) of the total variance. This QTL might be related to the QTL for grain yield mapped by Hayes et al. (1993).

One QTL for leaf angle was mapped on chromosome 4 (4H), but caution is required here as this linkage group had relatively few markers in this region. Sanguinetti et al. (1999) reported that among the ten QTLs found for leaf angle in maize, only one coincided with a QTL for leaf ABA-concentration.

In conclusion, for several traits at least one QTL was mapped. Most studies map QTLs for physiological traits under different stress treatments and usually find several QTLs for each trait. However, in this study QTLs were mapped under near-optimal growth conditions. Often stress situations accentuate differences already found under non-stressed conditions, resulting in higher LOD-scores at the position of the QTL (Bettey et al. 2000). This means that sometimes QTLs for traits remain undetected in unstressed conditions.

In the present study we used a *H. spontaneum* $\times$ *H. spontaneum* cross, which resulted in a relatively small number of markers that are common to the consensus *H. vulgare* map. Together with the low rate of polymorphism detected with SSR markers used in *H. vulgare* maps, the scope for conclusions about QTLs found in this study and mapped candidate genes in cultivated barley is very limited. However, we are the first to map QTLs for RGR and many of its underlying traits. Also the overlapping QTLs of photosynthetic rate and stomatal conductance improve the physiological insight in

already mapped photosynthesis-related traits. Further work is required to improve the map with more markers related to other published maps in barley, but also to determine the precise location of the QTL by fine mapping (Han et al. 1999).

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Chapter 5

Association of AFLP markers with growth-related traits in *Hordeum spontaneum*

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Abstract

In this paper the quantitative trait loci (QTLs) as identified within a specific cross between two wild barley (*Hordeum spontaneum* C. Koch) populations (Chapter 4) are compared with marker-trait associations assessed across a set of 81 wild barley accessions. The accessions were measured for 31 traits related to growth, C and N economy as well as plant morphology, for plants grown under close to optimal conditions. The accessions were genotyped for 70 AFLP markers, mapped earlier in cultivated barley as well as in wild barley. Of the 70 markers 63 had significant correlations to one or more traits. Several QTLs were supported by marker/trait associations. The QTL mapped on chromosome 1 (7H) for relative growth rate was supported by a marker/trait association in the middle of the interval for the QTL-location. Four markers related to photosynthetic rate per unit leaf area were mapped on chromosome 4 (4H), where also

the QTL for photosynthetic rate and stomatal conductance were found. One of them was also related to chlorophyll content, suggesting that this location on chromosome 4 (4H) might be very important for photosynthesis-related traits. Specific leaf area differed little amongst the 21 populations and therefore only one genetic marker was associated with this trait. On chromosome 1 (7H), 2 (2H) and 4 (4H) markers were strongly associated with leaf thickness, leaf width and seed mass, suggesting that these traits are genetically linked.

Introduction

Plant species differ considerably in their potential relative growth rate (RGR, net increase in biomass per unit biomass already present per unit of time) with plant species from fertile habitats showing much higher RGRs under optimal conditions than those from infertile habitats (Grime & Hunt 1975; Poorter 1989). These inherent differences in RGR are mainly due to variation in specific leaf area (SLA, the leaf area per unit leaf mass) and to a lesser extent to the leaf mass fraction (LMF, leaf mass per unit of plant mass) (for a review see (Poorter & van de Werf 1998)). A range of physiological, chemical and morphological traits are correlated with a plant's potential RGR (Lambers & Poorter 1992). To what extent these growth traits are genetically linked is still unknown. To answer this question the knowledge of interspecific differences in RGR and its underlying components is not of much help, because species differ largely in their genetic background. An approach where one species is studied, with a large genetic as well as phenotypic variation in growth traits, would be more useful. Wild barley (*H. spontaneum*), the progenitor of cultivated barley is widely distributed in the Fertile Crescent (Zohary & Hopf 1988) and occupies a wide range of different habitats (Nevo et al. 1979). Genetic diversity is present between populations collected from around the Fertile Crescent (for a review see Nevo (1992),Forster (1999)). The species also shows large morphological and physiological variation which can be related at least partly to the ecogeograpical origin of the populations studied (Nevo et al. 1983; Nevo et al. 1984; Nevo et al. 1986; Forster et al. 2000). The variation in physiological and morphological growth traits within and amongst populations in Israel has been determined under standardised, controlled conditions (Van Rijn et al. 2000). Two accessions of these populations, contrasting in growth- related traits, were used in a cross and an AFLP marker map was constructed (Vanhala et al., unpublished results). Growth-related traits were determined on the offspring (F_3-lines) and quantitative trait loci (QTL) were mapped (chapter 4). QTL mapping is a useful tool in locating genomic regions underlying the expression of phenotypic traits in fields like plant breeding and plant physiology (e.g. Quarrie et al. (1997), Teulat et al. (1997), Yin et al. (1999a) and This et al. (2000)). QTL analysis commonly is performed

using the simultaneous segregation of markers and traits within a cross between two contrasting lines of an agronomic plant species. Lately, QTL mapping in natural populations has become an interesting option, since it harbours a source of interesting, little exploited genome areas. Also in wild barley QTLs for several physiological traits, such as nitrogen content per plant mass, δ^{13}C, δ^{15}N (Ellis et al. 1997) (for a review see Forster et al. (2000)) and recently also for growth-related traits, such as RGR, SLA, ULR and photosynthetic rate (chapter 4).

An alternative way of linking genetic marker diversity to trait diversity is analysis of marker/trait associations in a set of accessions or populations. This is done by way of a regression analysis between molecular markers, such as AFLP, RAPD or SSRs on the one hand, and phenotypic data on the other (Forster et al. 2000). Pakniyat et al. (1997) described 39 wild barley genotypes and association between traits involved in salt tolerance and AFLP markers. They found 5 AFLP markers to be associated with salt tolerance, of which 3 could be mapped. Forster (1999) described SSR variation in 29 wild barley lines from Israel and mapped 11 SSRs. One of those was located in the Rubisco activase gene (*Rca*), and was found to be correlated with site- of-origin water availability.

Using a set of wild barley lines, accessions or populations instead of a mapping population of a cross between two parents has a number of advantages. First, there is no need to develop a range of recombinant inbred lines, which often take years to develop. Second, the observed associations are not limited to a single cross (Kraakman et al. 2000), which enables evaluation of a much wider germplasm. A third, related advantage is that screening of a wider range of genotypes minimizes the risk run in QTL experiments that both parents by chance have the same allele for a number of traits (Kraakman et al. 2000). However, this approach also has clear caveats. The problem is that several mechanisms, such as founder effects, genetic drift, population admixture, or selection, can generate linkage disequilibrium (Jannink et al. 2001). This may cause spurious associations between marker and phenotype in populations with several polymorphic loci, where alleles at two loci occur together more often than expected. This is especially a problem in selfing species such as *H. spontaneum,* as linkage disequilibrium is more extensive in such cases (Nordborg 2000). A high degree of homozygosity reduces recombination and therefore complicates the determination of the adaptive significance of a particular trait or gene (Eckardt 2001).

Notwithstanding these caveats, there is scope for application of this method. In our opinion, best insight is to be gained by combining the results of the marker/trait associations with those of a QTL analysis. In this way we can see which of the observed QTLs are supported by additional evidence, based on a range of different populations. Therefore AFLP markers were associated with the variation in growth traits, earlier found by (Van Rijn et al. 2000) in *H. spontaneum* populations and compared with the

QTLs found in chapter 4. Some of the AFLP markers are common to a *H. spontaneum* $\times$ *H. spontaneum* map (Vanhala et al., unpublished results) and some are common with a *H. vulgare* map (Qi et al. 1998).

Material and Methods

Plant material

Eighty-one wild barley (*H. spontaneum* C. Koch) accessions grouped in 21 populations from a wide range of locations in Israel, were used in this study. The populations along with available climatic data of the locations are listed in Van Rijn et al. (2000).

Seeds were germinated on moistened filter paper in Petri dishes in a refrigerator at 6°C and an irradiance of 10 μmol m^{-2} s^{-1}. After one week seedlings were transferred to a container with drainage holes which was filled with clean white beach sand. The sand was saturated with half strength of the following nutrient solution: 603 μM $Ca(NO_3)_2$, 795 μM KNO_3 , 190 μM KH_2PO_4, 270 μM $MgSO_4$, 0.2 μM $MnSO_4$, 0.9 μM $ZnSO_4$, 20 μM H_3BO_3, 0.3 μM Na_2MoO_4, 40 μM Fe-EDTA, 40 μM $FeSO_4$ and 47 μM SiO_2. The container was placed in a growth room for five days in the following conditions: 14/10 h day/night, 20°C day/night, irradiance of 450 $\pm$ 25 μmol m^{-2} s^{-1}, relative humidity 70%. Thereafter seedlings were transferred to 33 L tanks containing the nutrient solution described above, aerated and at full-strength, which was replaced weekly. The pH of the nutrient solution was adjusted regularly to 5.8 with H_2SO_4. To avoid mutual shading, the number of plants on each container ranged from 18 and 6, depending on the size of the plants. Plants were rotated four times a week within the growth room.

DNA isolation and AFLP analysis

Four to ten plants were grown per accession for DNA isolation. Leaves were collected from two-week old plants. The samples were stored at -80°C. DNA was isolated from 200-300 mg of frozen leaves according to the CTAB protocol (Saghai-Maroof et al. 1984).

The AFLP protocol was used as described in Vos et al. (1995). The DNA was digested with restriction enzymes *Eco*RI and *Mse*I. The AFLP bands were visualised by radioactive [γ^{33}P]-ATP labelling. The presence or absence of AFLP fragments was scored by eye (dominant scoring), and all clear bands with lengths between 80 and 500 nucleotides were included. In total 10 primer combinations previously tested with *H. vulgare* ssp. *vulgare* (Qi & Lindhout 1997) were used: E32M61, E33M55, E35M48, E35M55, E35M61, E38M54, E38M55, E39M61, E42M51, E45M55. Only markers that were mapped in a cultivated barley cross 'L94 $\times$ Vada' (Qi et al. 1998) and in a

wild barley cross 'Ashqelon × Mehola' (T.K. Vanhala et al. unpublished) were taken into the analysis.

Experimental design

Of each population, plants from four accessions (the progeny group from a single plant collected in the field) were grown. Of each accession eight plants were used to measure a range of traits, somewhat arbitrarily categorised in three groups related to growth analysis, C and N-economy and to morphology. Plants were measured at 23-25 days after germination, when there were 2-10 tillers. Whole-shoot photosynthesis and shoot and root respiration were measured on two plants of each accession. Fresh and dry mass of leaves, stems and roots, leaf area, leaf width, leaf angle, shoot height and the number of leaves and tillers were also determined on these two plants as well as on two additional plants. Two other plants were used for measurements of osmotic potential and to determine the chlorophyll concentration and the remaining two plants were used for measurement of leaf thickness. The latter four plants were also used for chemical analyses. Because of the large number of plants and the time required for the physiological measurements we staggered the germination and growth of the plants. Four randomly chosen accessions of different populations were measured each week.

Measurements

Seed mass of each seed was determined separately. Leaf width and thickness were measured on the youngest fully expanded leaf. Leaf angle was assessed as the average angle of the four oldest leaves. Plant height was determined by measuring total shoot length.

Leaf area, fresh and dry mass of the leaves (leaf blades), stems (leaf sheaths) and roots were determined to calculate water content ((fresh mass - dry mass) / dry mass) of leaf, stem and root (WCleaf , WCstem, WCroot, respectively), leaf area ratio (LAR, leaf area per total plant dry mass), specific leaf area (SLA, leaf area per leaf dry mass), leaf mass fraction (LMF, leaf dry mass per total plant dry mass), stem mass fraction (SMF, stem dry mass per total plant dry mass) and root mass fraction (RMF, root dry mass per total plant dry mass).

Carbon concentration and nitrogen concentration were determined with an elemental analyser (Carlo Erba 1110, Milan, Italy). Nitrate concentration was quantified according to Cataldo et al. (1975). Ash and ash alkalinity were determined as described by Poorter & Villar (1997). Results were used to calculate concentrations of organic acids, minerals and organic nitrogen compounds. The osmotic potential of the leaf sap was measured using a Wescor Vapour Pressure Osmometer (Logan, UT, USA).

The chlorophyll concentration of the leaf was determined according to Lichtenthaler & Wellburn (1983) after extraction with 80% acetone.

Net photosynthesis, dark respiration and root respiration were measured as CO_2 exchange. CO_2 and H_2O exchange were measured differentially to calculate photosynthesis per unit leaf area and per unit leaf mass, shoot respiration, root respiration and water use efficiency.

Further details on the phenotypic analyses are given in Van Rijn et al. (2000).

Calculations and statistical analyses

Relative growth rate (RGR) was estimated, on the basis of a plant's C-economy, using the formula given in Poorter & Pothmann (1992b):

$$\mathrm{RGR} = \frac{\mathrm{PS_A} \times \mathrm{SLA} \times \mathrm{LMF} - \mathrm{ShR} \times (\mathrm{LMF} + \mathrm{SMF}) - \mathrm{RR} \times \mathrm{RMF}}{\mathrm{C}} \qquad (5.1)$$

where, PS_A = daily rate of photosynthesis per unit leaf area, ShR = daily shoot respiration, RR = daily root respiration, SLA = specific leaf area, LMF = leaf mass fraction, SMF = stem mass fraction, RMF = root mass fraction and C = carbon concentration of the plant biomass). We only determined the carbon content of the leaves, assuming that it is representative of that of the whole plant. In reality, the carbon content of roots and shoots tend to be slightly lower than in leaves (Poorter & Bergkotte 1992a), but these differences are not likely to affect the RGR-calculation to more than a small extent. A second assumption is that the rates of photosynthesis and respiration, both measured over a two-hour period, can be integrated over 24 hours. All parameters of the RGR-formula were measured on the same day.

To study marker-trait associations, simple product moment correlations were calculated (Pearson correlations) between a selected set of markers and the 31 quantitative traits described earlier. Markers were included on the basis of membership of either 'Ashqelon × Mehola' or the 'L94 × Vada' map. Of the original 643 polymorphic markers, 70 retained. A test for the correlation is in this case equivalent to a two-sample t-test comparing the mean of the accessions with a specific marker band with the mean of the accessions without that marker band. As levels of test a liberal 0.05 was chosen, besides a more strict 0.0007 (0.05 divided by the number of selected markers), the latter based on a Bonferroni correction for simultaneous testing. All the calculations were performed using SAS (SAS system for Windows 6.12, 1989-1996 SAS Institute Inc. Cary, NC, USA).

Results

Of the 70 markers, 63 correlated significantly ($p < 0.05$) with at least one of the measured traits. For these 63 markers in total 274 marker-trait correlations were observed at the 0.05 significance level. At the strict significance level of 0.0007, 33 marker-trait correlations were found significant. The strongest correlation was found between seed mass and a marker from chromosome 4 (37.6 cM in 'Ashqelon × Mehola' map) (r=-0.70). Figure 5 shows the results of the marker-trait correlations positioned on the 'L94 × Vada' and 'Ashqelon × Mehola' maps. The QTLs from chapter 4, are also added for comparison.

Mapped QTLs supported by marker-trait associations

Nine QTLs were clearly supported by at least one marker-trait correlation at the 0.05 significance level (Table 5.1). At the strict 0.0007 significance level one marker-trait correlation (leaf width with a marker at chromosome 4, 50.9 cM in 'L94 × Vada' map) supported a QTL at the same position. Supportive marker/trait associations are indicated as grey boxes in Fig. 5.

Mapped QTLs not supported by marker-trait associations

There were 18 QTLs that were not clearly supported by the marker-trait correlations. In six cases significant marker-trait correlations were detected at the same chromosome as the corresponding QTL, but not in the actual position of the QTL. In two of these cases (seed mass, chromosome 2 (2H), 7.8 cM 'L94 × Vada' map, and PS_M chromosome 5(1H), 111.5 cM 'L94 × Vada' map) the correlation (r=-0.46 and r=-40, respectively) was highly significant ($p<0.0001$ and $p<0.0004$, respectively). None of the sub-significant QTLs described in chapter 4 was supported by marker- trait correlations.

Figure 5.1 (next pages): The significant marker-trait correlations among *H. spontaneum* accessions and QTLs for 29 traits in a *H. spontaneum* cross. The 63 markers were based on two linkage maps; 'L94 × Vada' (Qi et al. 1998) and 'Ashqelon × Mehola' (Vanhala et al., unpublished). The arrows point to the map-based marker positions. The marker-trait correlations are shown on the left side of the chromosomes. The traits that correlate with a marker at the 0.0007 significance level are shown in boldface, and those that correlate at the 0.05 significance level are shown in italics. The traits that correlate with a marker and support a QTL are shown in grey boxes. The confidence intervals of the detected QTLs are given on the right side of the 'Ashqelon × Mehola' map. The common markers shared between the two maps are shown with connecting lines. Dashed lines are used to point to the approximate positions of the SSR markers in the 'L94 × Vada' map.

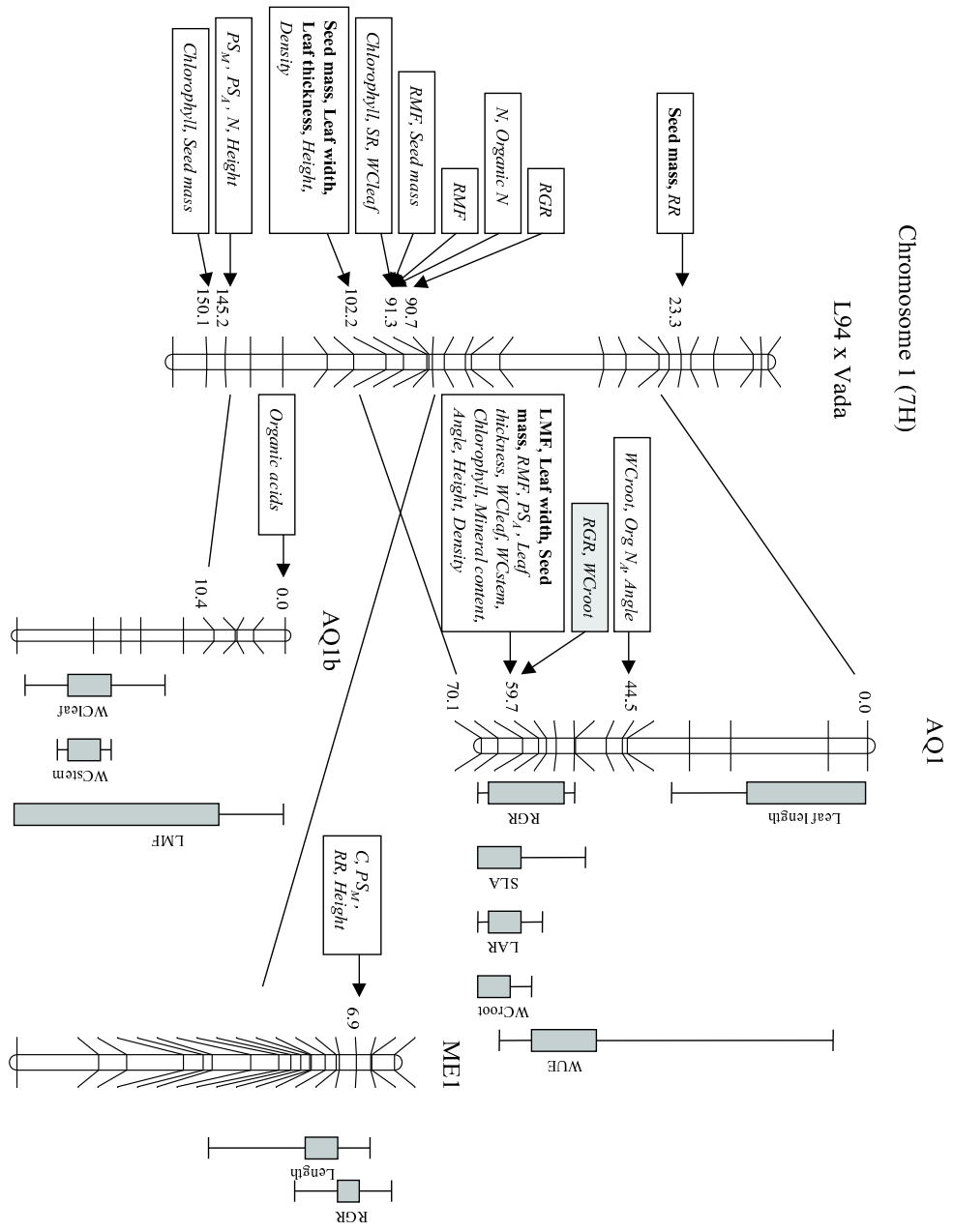

Figure 5.1: continued.

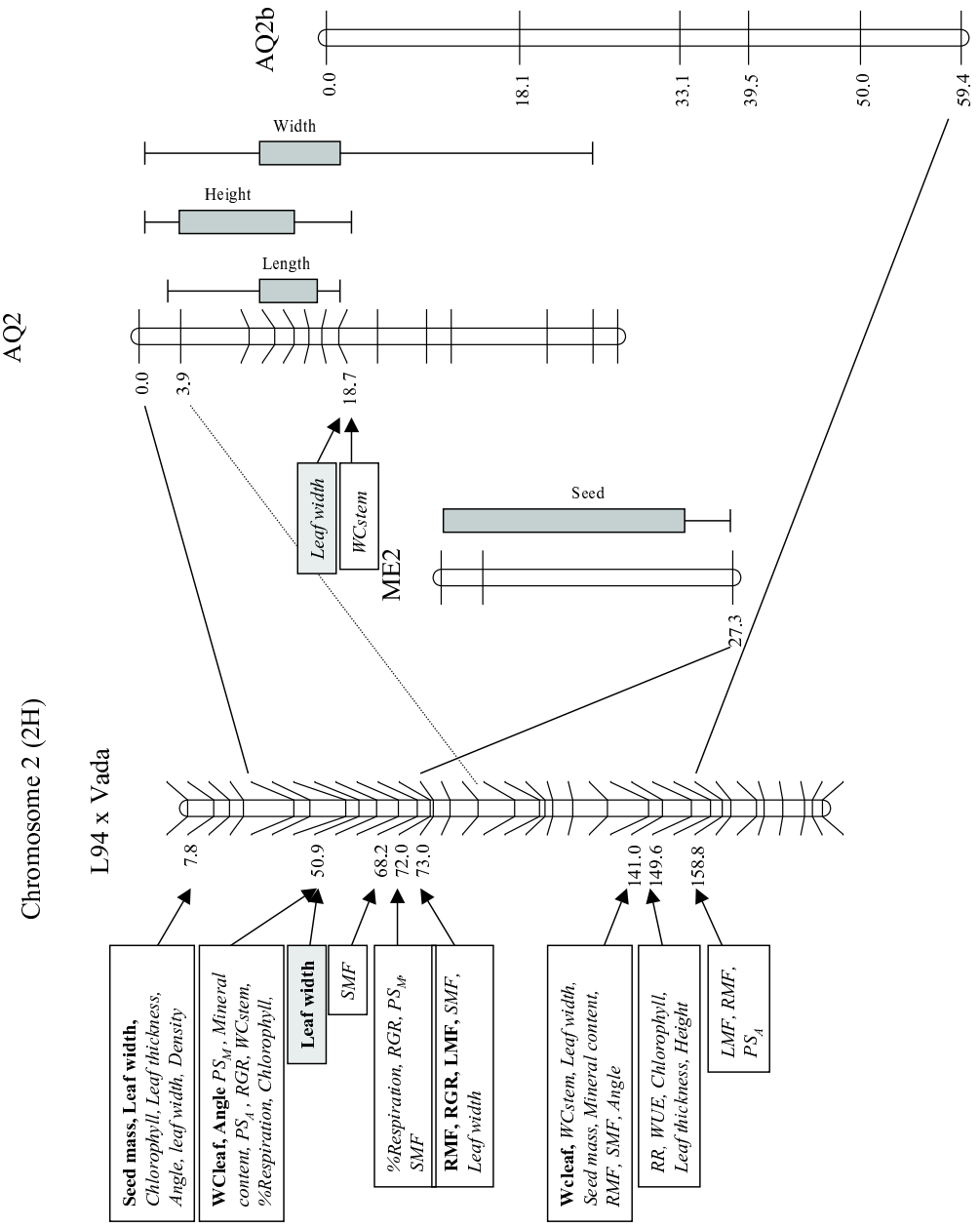

Figure 5.1: continued.

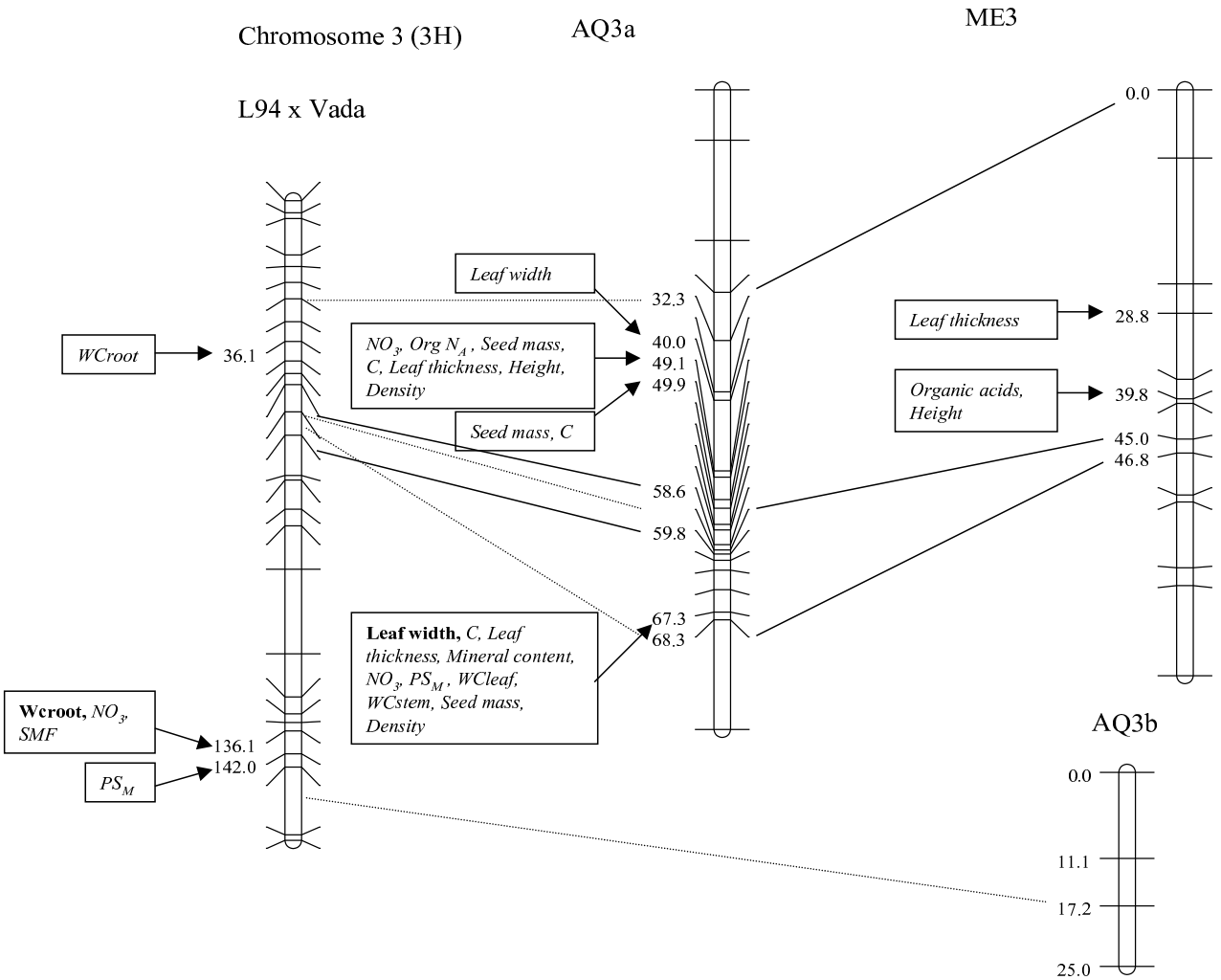

Figure 5.1: continued.

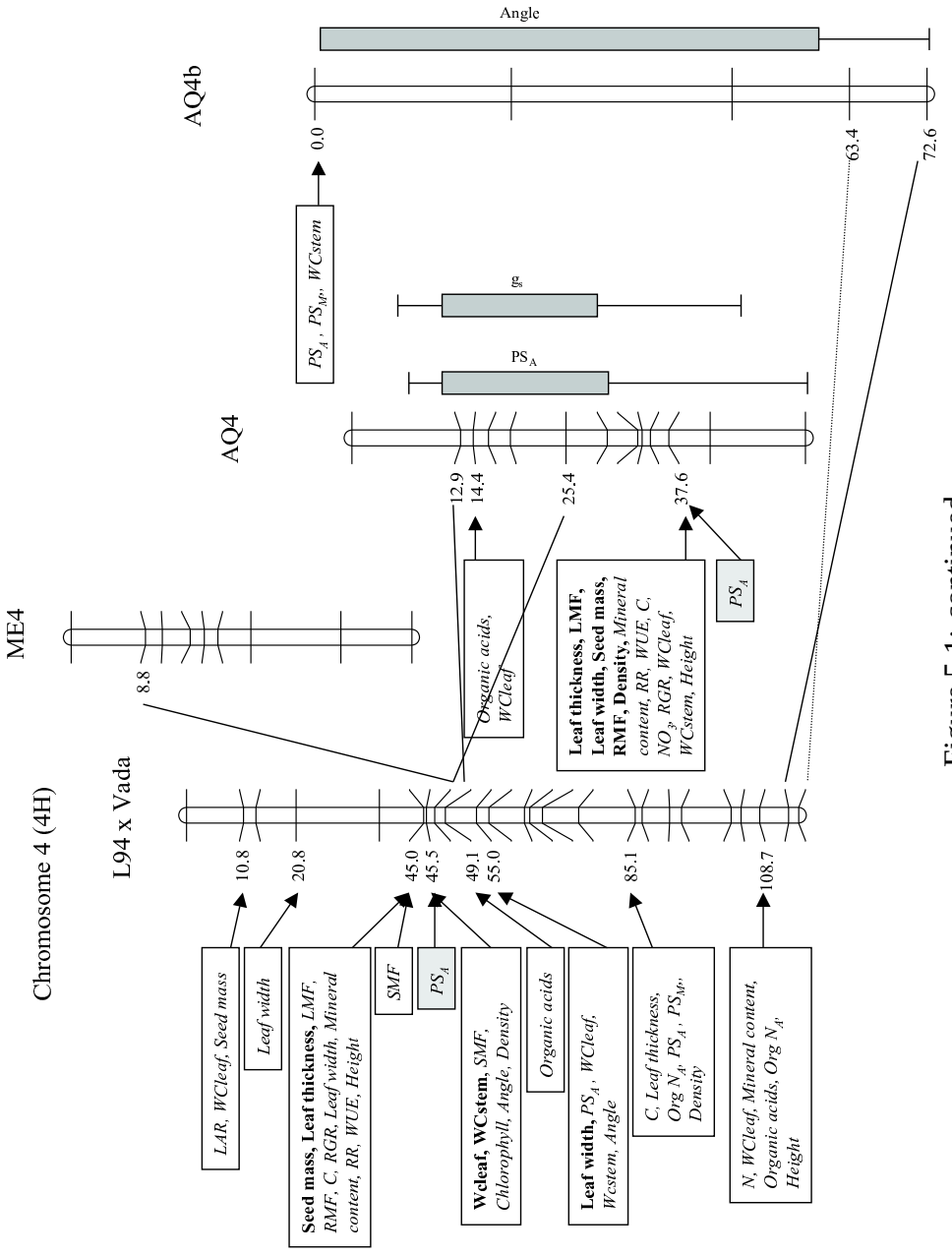

Figure 5.1: continued.

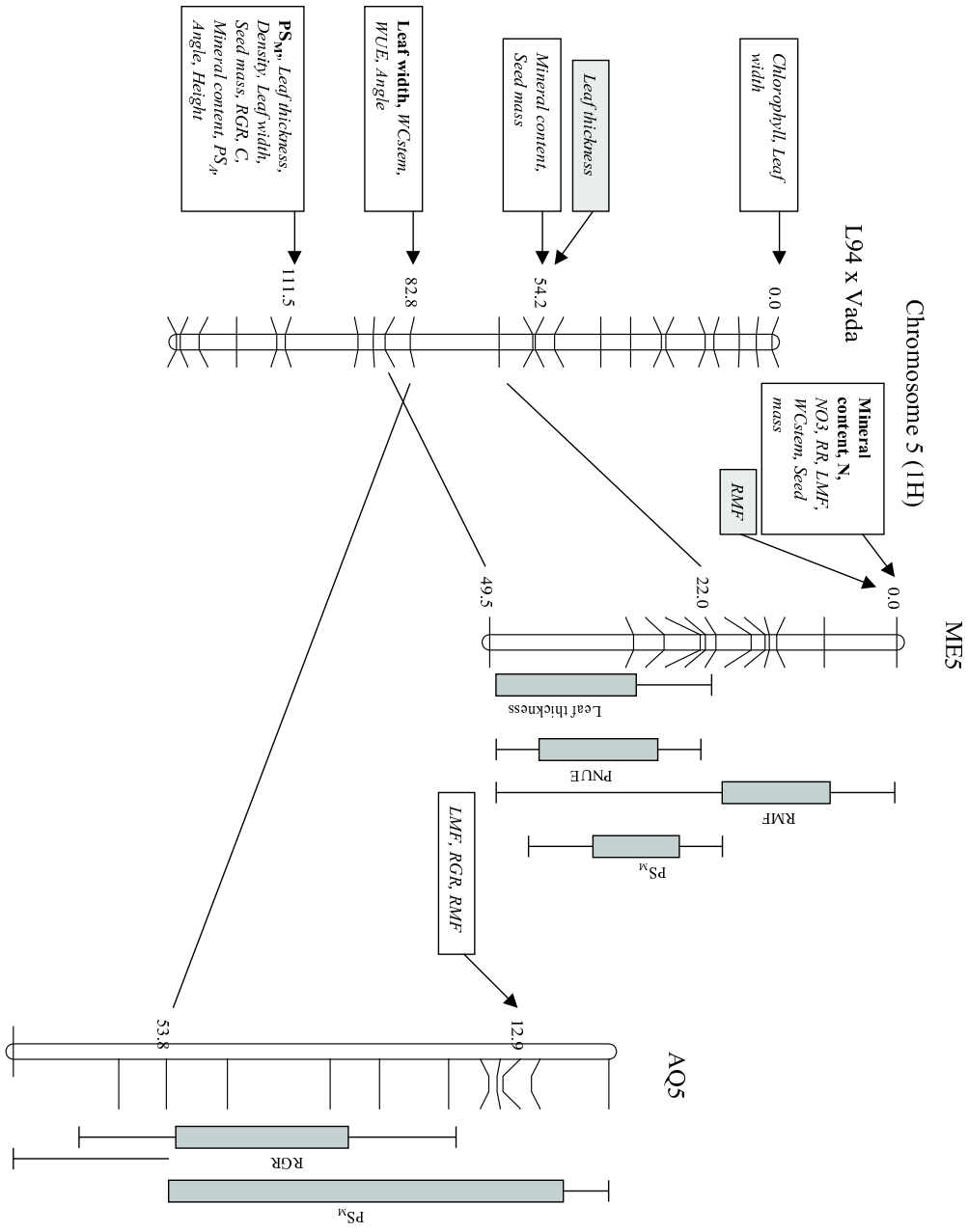

Figure 5.1: continued.

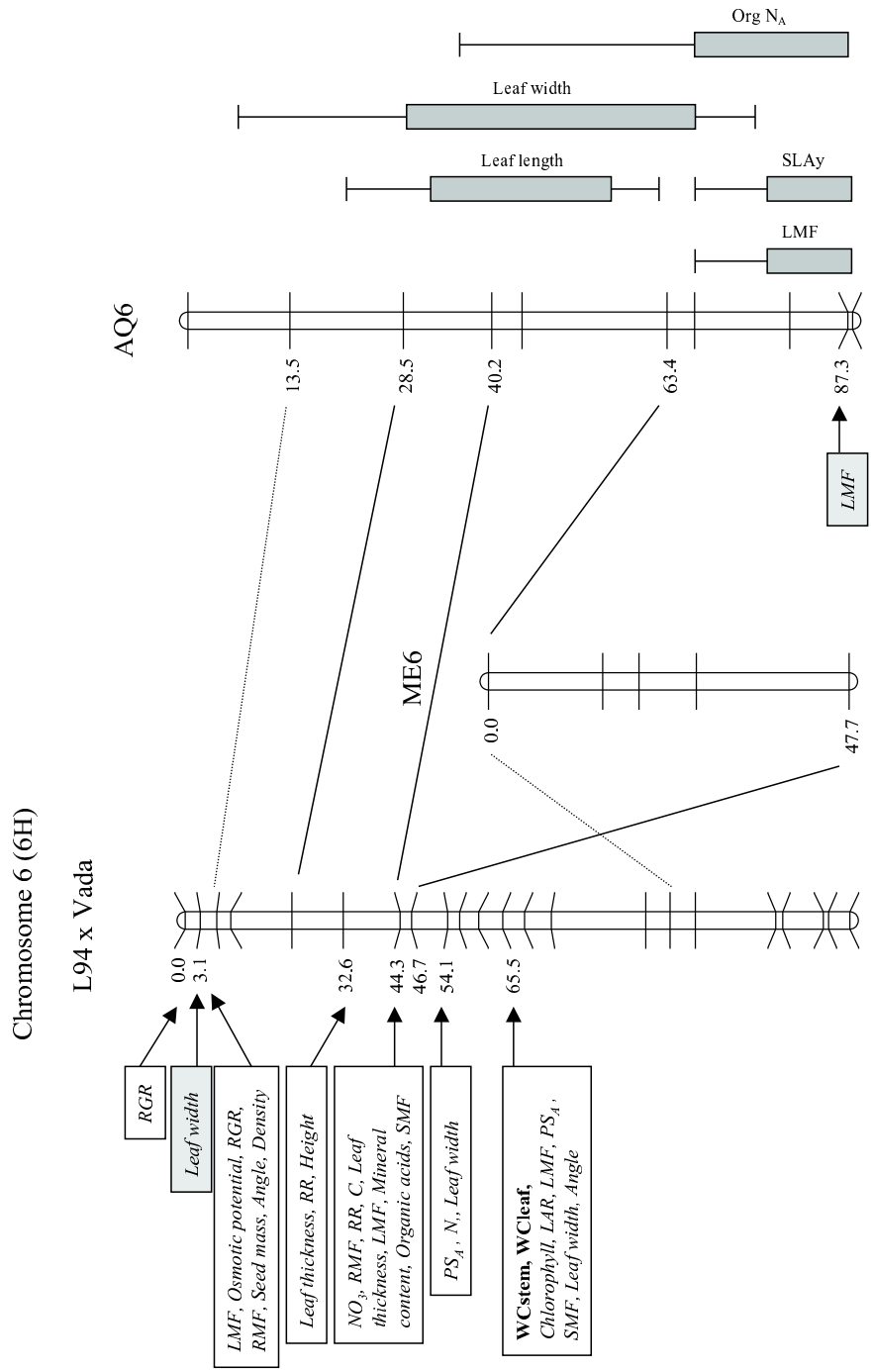

Figure 5.1: continued.

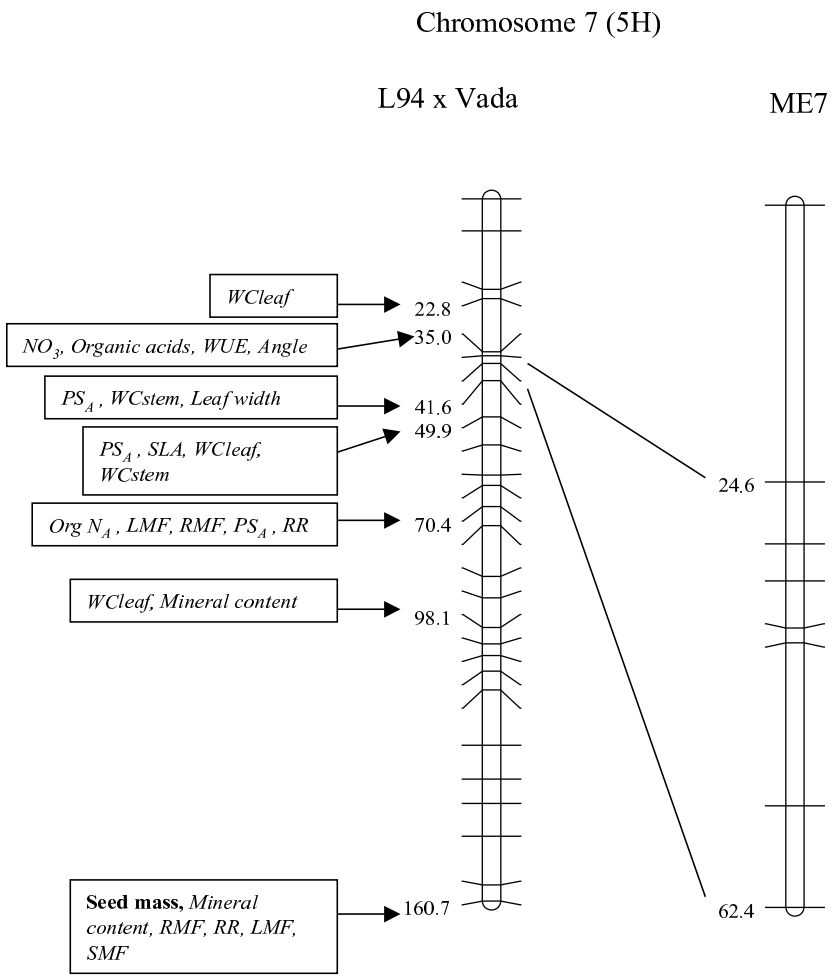

Figure 5.1: continued.

Trait	Number of marker-trait correlations*		QTLs detected	Number of QTLs supported*	
	P<0.05	P<0.0007		P<0.05	P<0.0007
Leaf Angle	12	1	1		
C	9	0			
Chlorophyll	9	0			
Leaf mass density	9	1			
Height	14	0	1		
LAR	2	0	1		
Leaf thickness	14	3	1	1	
Leaf width	20	4	2	2	1
LMF	14	3	1	1	
Mineral content	13	1			
N	5	1	1	1	
NO_3	8	0			
Organic acids	7	0			
Organic N_A	9	0			
Osmotic potential	1	0			
PS_A	15	0	1	1	
PS_M	9	1	1		
%Respiration	2	0			
RGR	11	1	3	1	
RMF	14	2	1	1	
Root respiration	10	0			
Seed mass	18	7	1		
SLA	1	0	1		
SMF	10	0			
Shoot respiration	0	1			
WCleaf	15	4	1		
WCroot	4	1	1	1	
WCstem	14	2	1		
WUE	5	0	1		
Total	274	33	20	9	1

*The numbers at 0.0007 significance level are included in the columns for p<0.05.

Table 5.1: The 29 traits measured in 81 wild barley accessions, the number of marker- trait correlations at 0.05 and 0.0007 significance levels, number of QTLs detected in an earlier study (see Chapter 4) and the number of supported QTLs by one or more marker-trait correlations at the 0.05 and 0.0007 significance levels.

Other marker-trait associations

The strongest correlations with genetic markers were observed for morphological traits such as seed mass and leaf width or thickness. RGR also showed a strong correlation with a marker on chromosome 2 (2H) together with two of its components, LMF and RMF. On chromosome 5 (1H), several chemical traits were highly significant as well as the rate of photosynthesis per mass and leaf width. Chromosomes 3 (3H), 6 (6H) and 7 (5H) contained only few strong marker-trait correlations whereas chromosomes 1 (7H), 2 (2H) and 4 (4H) contained many highly significant associations.

Discussion

Variation in RGR and its underlying components have been studied extensively, but to what extent these traits are genetically linked and/or caused by common factors is unknown. Therefore we have carried out a QTL analysis, in order to determine the place of control of these traits and to assess the overlap of some traits on the genome (chapter 4). In the present study we want to compare the QTLs found for growth- related traits with the marker/trait associations found in 81 accessions of *H. spontaneum*. The AFLP analysis determined 643 polymorphic bands between the 81 accessions. We will only focus on 70 AFLP markers, because these markers could be linked either to the 'Ashqelon $\times$ Mehola' map (Vanhala et al., unpublished results) or the 'L94 $\times$ Vada' map (Qi et al. 1998). A number of linkage groups in the 'Ashqelon $\times$ Mehola' map could be assigned to a specific chromosome due to markers identical with those found for *Hordeum vulgare*. However, in some cases there was only one common marker, which complicates the comparison between the two approaches. This is because a marker/trait association could or could not fall into an observed QTL region, depending on how this linkage group would align to the 'L94 $\times$ Vada' map. In those cases we choose the most conservative option, considering it as no evidence for a QTL.

Marker/trait associations for growth-analysis traits

A total of eleven markers was associated with RGR. The marker that showed the strongest correlation with RGR explained 15% of the variation and was mapped on chromosome 2 (2H). One of the other markers that correlated with RGR was located in the middle of the interval for the location of a QTL mapped for RGR on chromosome 1 (7H) and explained 11 % of the total variation. For the other traits with QTLs in this same region no marker-trait correlations with this 'RGR-marker' were found, with the exception of WCroot. However, some other interesting traits, such as PS_A, biomass allocation to leaves and root, water contents of all organs and seed mass, were also correlated with this marker. This is interesting, as often no correlation is found be-

tween PS_A and RGR in interspecific studies (Mooney et al. 1978; Delucia et al. 1989; Poorter 1989), with the exception of shade species compared to sun species under high light (Pons 1977), where positive correlations are found.

The QTL for RGR mapped on chromosome 5 (1H) was not supported by markers that were associated with RGR. However, two flanking markers that were mapped outside the two-LOD support fall-off of the QTL were correlated with RGR. One of these markers was also associated with PS_M.

In many studies SLA is the most important trait explaining differences in RGR (see for a review Poorter & van de Werf (1998)). However, there was almost no difference in SLA in the 81 accessions (Van Rijn et al. 2000), and no significant difference between the 21 populations. Only one marker was correlated with SLA, located on chromosome 7. In contrast with these results, the F_3 population showed substantial difference in SLA (range from 33 - 45 m^2 kg^{-1}) and a QTL was detected at the same location as the QTL for RGR. Clearly, the results for the specific cross are different from those of the populations. The QTL for LMF mapped on chromosome 6 (6H) was supported by a marker that was also linked to LMF. The marker on chromosome 4 (4H) that was linked to LMF was a neighbouring marker of the position of a subsignificant QTL for LMF. Two markers were strongly correlated with LMF, one on chromosome 1 (7H) and one on chromosome 4 (4H). The marker on chromosome 1 (5H) was the same one that was linked to RGR. LMF is usually not the most important factor explaining differences in RGR, but is sometimes positively correlated with RGR (Ingestad 1981; Poorter & Remkes 1990), sometimes negatively (Hunt et al. 1987; Shipley & Peters 1990).

Marker/trait associations for C and N economy-related traits

Four markers that were correlated with PS_A were mapped on chromosome 4 (4H), where also the QTL for PS_A was mapped. These four markers together explained 32% of the total variation, whereas the QTL explained 20%. One of these markers appeared on the Ashqelon $\times$ Mehola map and was located in the support interval of the QTL for PS_A, the other markers were contained in the L94 $\times$ Vada map. Two common markers in this region gave a good indication that these three markers are located within the QTL for PS_A. Note that photosynthesis was determined differently in both studies. In the QTL study photosynthesis was measured on a single leaf, in this study photosynthesis was determined on the whole plant. The fact that both measurements point to the same direction makes these observations relatively robust

Another interesting detail is that also a QTL for chlorophyll content was found on chromosome 4 (4H) in barley (This et al. 2000). In the QTL study chlorophyll content was not measured, but in the population study we did and the marker that showed

the best correlation with chlorophyll content was also located on chromosome 4 (4H). These results, together with the mapping of a Rubisco activase gene on chromosome 4 (4H) in *H. spontaneum* (Becker & Heun 1995) support again that this location on chromosome 4 (4H) might be very important for area-based photosynthetic traits.

Marker/trait associations for morphological traits

The QTL for leaf width on chromosome 2 (2H) is supported by a marker strongly correlated with leaf width. The other QTLs on this chromosome overlapping with the QTL for leaf width were plant height and leaf length. These parameters were not measured in the population study. On chromosome 4 (4H) one marker is strongly correlated with several morphological traits, such as leaf thickness, leaf width and seed mass and the biomass allocation to leaves and roots. On chromosome 2 (2H) another marker is also strongly correlated with leaf width and seed mass. On chromosome 1 there is also a marker mapped, which is strongly related to leaf thickness, leaf width and seed mass. These results suggest that these morphological traits are at least genetically linked, but maybe controlled by a common factor. Since on chromosome 2 (2H) (Börner et al. 1999) as well as on chromosome 4 (4H) (Ivandic et al. 1999) *dwarfing* genes are mapped in barley, a GA controlled gene might be a likely candidate. However, these genes were mapped in cultivated barley and this does not necessarily mean that those genes are also present in *H. spontaneum*. The two QTLs for WCleaf and WCstem were not supported by marker/trait associations, also because no markers were mapped in this area. The QTL for seed mass on chromosome 2 (2H) was not supported by marker/trait associations either, although one marker on chromosome 2 (2H) was strongly correlated with seed mass.

Conclusions

Several QTLs mapped in the former study are supported by marker/trait associations in 21 wild barley populations. The QTL detected on chromosme 1 (7H) for RGR was supported by an association of a marker with RGR. However, no association with SLA was found at this marker position. Markers associated with PS_A and chlorophyll content were mapped on chromosome 4 (4H), where also QTLs for PS_A and stomatal conductance were found. Many markers are associated with leaf thickness, leaf width and seed mass, suggesting that these traits are tightly linked.

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Chapter 6

Summarizing discussion

Introduction

When grown under near-optimal conditions plant species characteristics of nutrient-rich habitats show faster relative growth rate (RGR) than plants from nutrient-poor habitats (Grime & Hunt 1975; Poorter & Remkes 1990). Under these conditions, fast-growing species produce more leaf area per leaf mass, which contributes to a larger carbon gain per unit plant mass (Lambers & Poorter 1992). Photosynthesis per unit leaf area is not necessarily differed when fast- and slow-growing species are compared, due to an equal amount of biomass, which is 'diluted over a larger leaf area (Dijkstra & Lambers 1989b; Poorter et al. 1990). Fast-growing species also tend to have faster respiration rates, but when expressed as a fraction of the total amount of carbon fixed per day, they use less in respiration (Lambers & Poorter 1992). The genetic background of such correlations found between a species' relative growth rate and numerous growth-related traits is still unknown. In this thesis the main question is: To what extent are these growth-related traits genetically linked and/or caused by a common factor? To answer this question the present information about differences between species is not suitable. Therefore an analysis was performed amongst populations of a single species and crosses between them. *Hordeum spontaneum* C. Koch, the progenitor of cultivated barley (*H. vulgare* L.) was chosen for this study, because it occupies a wide range of different habitats and possesses a large genetic variation (Nevo et al. 1983; Nevo et al. 1984; Nevo et al. 1986; Corke et al. 1988; Nevo 1992).

Variation in growth characteristics in *H. spontaneum* populations, the genus *Hordeum* and the F_3-population of a cross between two *H. spontaneum* populations

As a first step in this research program the variation in growth characteristics of 21 populations of *H. spontaneum* from different sites in Israel was determined (Chapter 2). From each population four accessions were grown in a growth chamber under near-optimal conditions and a range of physiological, morphological, allocation-related and chemical growth characteristics were measured. RGR was estimated (Equation 2.1) as the carbon gain in photosynthesis minus the carbon loss in shoot and root respiration divided by the carbon concentration of the plant material already present. Averaged over all traits measured, the variation explained by differences amongst populations was 26%, amongst accessions 21% whereas that within accessions was 53% (Fig. 2.2a). As *H. spontaneum* is an inbreeding species, it was expected that the variation explained by differences within accessions was much lower. Maternal effects, environmental differences within the growth chamber and/or the influence of rare outcrossing rates (1.6% averaged over 26 populations (Brown et al. 1978b)) might be the cause of the large within-accession variation. When this variation was separated for the four groups of traits, it showed that the percentage of the total variation explained by differences within accessions for physiological traits was large that it and was the smallest in morphological traits (Fig. 2.2b). One of the reasons could be that the measurement errors involved in determining physiological traits are larger than those for morphological traits. Also the number of replicates was smaller for physiological traits than for morphological traits.

The variation explained by differences amongst populations was large in morphological traits (46%). The same conclusion could be drawn from the growth analysis amongst species in the genus *Hordeum* (Chapter 3). Nevo et al. (1984) showed that *H. spontaneum* populations are characterised by a large variation in morphological traits. The morphological trait that differed the most in all three studies (Chapters 2, 3 and 4) was seed mass. Amongst *Hordeum* species the difference was 25-fold and amongst the populations it was 4-fold.

The variation amongst *Hordeum* species was somewhat larger than within *H. spontaneum*. However, for most of the traits studied, the variation amongst the *H. spontaneum* populations was of the same magnitude as for the *Hordeum* species. This makes *H. spontaneum* a very interesting species for an analysis into the genetic basis of variation in growth-related traits.

RGR and SLA

Differences in RGR can be explained by differences in LAR or by differences in ULR. LAR is the product of SLA and LMF. ULR will be discussed in the following section. Poorter & van de Werf (1998)) concluded in a meta analysis of 111 studies that LAR and more specifically SLA is the most important factor explaining inherent variation in RGR. However, no significant variation in LAR and SLA was observed in a comparison of *H. spontaneum* populations (Chapter 2). One has to consider that this was a study within a species, which might have caused this little difference. Therefore growth traits were studied also amongst *Hordeum* species. In this experiment, which showed larger differences in RGR, the relation with LAR was significant, with the differences in LAR mainly due to variation in SLA (Chapter 3). There was a strong negative relation between ULR and LAR (Fig. 3.2). This was also true for 20 *Aegilops* species, grown in the same conditions (Villar et al. 1998). Two explanations have been offered for a negative relationship between ULR and LAR by Konings (1989) and Poorter (1989). Firstly, a higher LAR generally goes with a large leaf area per unit root mass. This could imply that water uptake is low compared to the transpiring surface, causing the water status of the leaves to be impaired and thereby the rate of photosynthesis. Alternatively, an increase in ULR may require an increase in photosynthetic rate, which can be realised by decreasing the SLA, so that the nitrogen content per unit area will be higher, and thereby photosynthetic capacity. As the negative correlation between ULR and LAR is mainly caused by a negative correlation between ULR and SLA, the second explanation is expected to be the most probable.

The two parents of the cross did not differ very much in SLA (Chapters 3 and 4), but their offspring in the F_3 population showed a substantial variation in SLA. One QTL was detected for SLA and was co-localised with a QTL for RGR on chromosome 1 (7H). Both QTLs had a negative additive effect, contributed by the Ashqelon parent (allele). This was also supported by the correlation analysis where RGR and SLA showed a strong positive correlation. On chromosome 5 (1H) the other QTL for RGR overlapped with a sub-significant QTL for SLA of the youngest full-grown leaf, with additive effects in the same direction. These results suggest that there might be a genetic basis for the positive relationship between RGR and SLA. A point of consideration is that RGR is a complex composite trait, which is probably controlled by several loci on the genome. Problems arise when QTLs only have a small individual contribution and are therefore more difficult to detect (Kearsey & Farquhar 1998b). The QTL analysis may provide few major QTLs with large effects, but may fail to detect a range of QTLs with small effects. The co-location of QTLs for RGR and SLA might be caused by two closely linked genes or by just one gene having an effect on both traits. One possibility is that the level of a hormone, such as gibberellin

or abscisic acid affects RGR and SLA. A positive correlation between RGR and endogenous GA concentration was found for maize by Rood et al. (1990). Application of GA increased RGR and SLA in *Plantago major* (Dijkstra et al. 1990), increased SLA in two *Aegilops* species (Bultynck 2001) and increased leaf expansion in bean (Brock & Cleland 1989) as well as in barley (Smith et al. 1996). Nagel et al. (2001) found that GA-deficient tomato mutants had a decreased RGR and SLA. Therefore, genes controlling the action of these hormones might be underlying the QTLs found for RGR and SLA. A possible mechanism could be that GA affects leaf expansion, which increases SLA and thereby causing an increase in RGR. Since there is not much information about where these genes might be located on the barley chromosome and since different markers are used in other studies it is as yet hard to focus on specific candidate genes. Growth-related traits have hardly been the subject of QTL-analysis. One of the few is the study of Causse et al. (1995) in which a correlation was found between invertase activity and the number of days required for leaf 3 to fully mature. The most probable explanation for the co-location of the QTLs found for RGR and SLA would be that in those regions several genes encoding enzyme activity (that might be under control of GA or ABA) are very tightly linked.

ULR and photosynthetic rate

ULR is a complex trait that represents the carbon gain in photosynthesis and the carbon losses in shoot- and root respiration and exudation as well as the carbon concentration of the plant Lambers & Poorter (1992). Amongst *Hordeum* species there was no significant correlation between RGR and ULR (Fig. 3.1a). In order to evaluate the relative importance of growth parameters in explaining variation in RGR, Poorter & van de Werf (1998) introduced the so-called growth response coefficient (GRC, the relative change in growth parameters such as ULR and LAR, scaled to the relative change in RGR). They found that, combined over a range of studies within a genus, ULR only explained 4% of the variation in RGR. In the growth study of the 16 *Hordeum* species ULR explained 38% of the variation in RGR. This value is closer to the average value (26%) that Poorter & van de Werf (1998) found for a compilation of 57 experiments. In Chapter 3 the GRC values of a study of 20 *Aegilops* species (Villar et al. 1998) was calculated and in that study ULR (54%) was even more important than LAR (46%) in explaining variation in RGR.

Photosynthetic rate is the most important trait explaining differences in ULR and is in general positively correlated with ULR (Konings 1989; Van der Werf et al. 1998; Evans 1998). This was also true for the F_3 population, where a strong positive correlation between PS_A en ULR was found (Table 4.2). No significant QTL was found for ULR, but the QTL with the highest peak LOD -score was overlapping with the

QTL for PS_A (Tables 4.2 and 4.3). In the marker/trait associations from 81 accessions, a marker on every chromosome was associated with PS_A (Table 5.1). The strongest correlations were with markers on chromosome 1 (7H), 2 (2H) and 4 (4H). The marker on chromosome 4 (4H) coincided with the QTL found for PS_A, explaining 20% of the total variation. On chromosome 1 (7H) this marker was associated with several growth traits, but also with RGR. The major QTL for RGR was also detected on chromosome 1 (7H), explaining 16% of the total variation. In the study of 21 populations of *H. spontaneum*, PS_A and RGR were positively correlated (Chapter 2, data not shown) but in the F_3 population they were strongly negatively correlated (Table 4.2). An explanation might be that PS_A in the former study was measured on the whole plant and from these measurements RGR was calculated and might therefore cause a positive correlation. In the latter study PS_A was measured as the rate of photosynthesis of a single leaf, and RGR was determined in a growth analysis. It might give a better perspective on growth when these two traits are measured independently. The strong negative correlation between the two variables is probably caused by the strong positive correlation between RGR and SLA. SLA is negatively related to leaf mass density and organic nitrogen per unit leaf area. This means that plants with a high RGR and SLA have a low photosynthetic rate per unit leaf area, caused by lower organic nitrogen per leaf area. There is often no correlation between RGR and PS_A (Poorter & Remkes 1990; Reich et al. 1992; Walters & Reich 1996).

The QTL for PS_A on chromosome 4 (4H) co-localised with a QTL for stomatal conductance (Fig. 4.3b). Interestingly, in *Helianthus annuus* several QTLs were mapped for PS_A and stomatal conductance, but none of them were overlapping (Hervé et al. 2001). A positive correlation between photosynthetic capacity and stomatal conductance has been described by Wong et al. (1979) and Körner et al. (1979). The rate of photosynthesis is dependent on the stomatal conductance and the difference in CO_2 inside and outside the leaf (Von Caemmerer & Farquhar 1981). The intercellular carbon dioxide concentration was the same amongst the F_3-lines (data not shown), as was also found in a comparison of 24 species differing in relative growth rate (Poorter & Farquhar 1994) as well as in 27 species native to the temperate forest region (Yoshie 1986). A reason to believe that the coincidence of the QTLs for PS_A and g_s is caused by one gene having an effect on both traits, is the mapping of the Rubisco activase gene (*Rca*) on a *H. spontaneum* map on chromosome 4 (4H) (Forster et al. 2000). CO_2-assimilation is mediated by Rubisco, and Rubisco activase is an enzyme, which activates Rubisco. Maybe Rubisco activase may control processes like photosynthetic rate and indirectly the stomatal conductance. Another interesting QTL, also found on chromosome 4 (4H) in barley, is the chlorophyll content per unit leaf area (This et al. 2000). In the marker/trait analysis four markers were found on chromosome 4 (4H) which were associated with PS_A (Fig. 5.1). Chromosome 4 (4H) might therefore be an

important chromosome for many traits related to photosynthetic capacity.

The other two components determining variation in ULR are the fraction of daily fixed carbon that remains in the plant and is not respired or exuded by the various organs of the plants (FCI) and the carbon concentration of the plant ([C]) (equation 1.2). Differences in the fraction of daily-fixed carbon incorporated between fast- and slow- growing species have been found, as fast-growing species respire relatively less of the daily carbon gain in photosynthesis (Poorter et al. 1990; Van der Werf et al. 1993). Some care has to be taken in the interpretation, as a number of assumptions were involved in estimating these traits. However, in the F_3 population both traits were not significantly correlated with ULR. Therefore it is concluded that these factors are of minor importance. These two traits did not differ to a large extent amongst the 21 populations or in the F_3 population.

The correlation between early biomass, RGR and seed mass

Differences in biomass can result from differences in seed mass, emergence time or variation in RGR (Van Andel & Biere 1990). Nevo et al. (1984) studied differences in biomass amongst populations originating from different habitats in Israel. When these populations were grown under favourable conditions in a garden experiment, biomass of populations from mesic sites was about twice that of populations from xeric sites. In our study of populations of *H. spontaneum* (Chapter 2) grown under near-optimal conditions similar results were obtained. However, these differences in biomass were not as much caused by differences in RGR (Fig. 2.4b), but largely due to differences in seed mass (Fig. 2.4a). This was also found in a study of *Hordeum* species (Fig. 3.3). This is in agreement with Chapin et al. (1989), where seed size was found to be more important than RGR, in determining variation in plant size (35 days after sowing) across four different *Hordeum* species grown under favourable nutrition. Also in a study to determine the major factors that are responsible for variation in early vigour in barley, wheat and oat, embryo size was found the most important (López-Castañeda et al. 1996).

No correlation between seed mass and RGR was found in the between-population study (Chapter 2) or the amongst species study (Chapter 3). Seed size usually correlates negatively with RGR in interspecific studies across genera (Shipley & Peters 1990; Jurado & Westoby 1992; Marañón & Grubb 1993; Reich et al. 1998). In intraspecific studies this relationship is not found (Clevering 1999) or a positive relationship is observed (Meerts & Garnier 1996). However in the F_3 population a strong negative relationship between RGR and seed mass occurred (Table 4.2). One QTL, on chromosome 2 (2H), was detected for seed mass, which explained a large part of the total variation (29%), but was not overlapping with any other QTL (Fig. 4.3a). A sub-

significant QTL for seed mass was found on chromosome 5 (1H) which overlapped with a QTL for RGR, with opposite additive effects. Three markers on chromosome 1 (7H) were strongly associated with seed mass. One of them was located on the position of the QTL for RGR (Fig. 5.1). This may imply that also the relationship between seed mass and RGR might have a genetic basis.

The correlation of growth traits with environmental factors

In all experiments described in this thesis plants were grown under favourable conditions with an ample supply of nutrients and water. This might be a reason for the poor correlation between growth traits and environmental factors amongst 21 *H. spontaneum* populations. Although environmental data of the natural habitat of the populations (Chapter 2) and the species (Chapter 3) was rather poor, an attempt was made to determine the correlation between growth traits and environmental factors. Most traits were not related to any environmental factor at the site of origin of the 21 populations. Photosynthetic rate was faster for plants from xeric environments than for plants from mesic environments (Chapter 2). Also, plants from xeric habitats had smaller leaves, a lower leaf angle and more tillers. Narrower leaves might help reducing the transpiration in dryer environments (Von Willert et al. 1992). A lower leaf angle means that plants have a higher light absorption and that might explain the higher rate of photosynthesis. For *Hordeum* species it seems that the potential RGR decreases with increasing altitude in the natural habitat. *H. comosum* and *H. brevisubulatum* occur at high altitudes in the Andes in South America and at high altitudes in Asia, respectively (Von Bothmer et al. 1995) and both show low RGR values (Chapter 3). The parents of the cross, showed also differences that can be somehow related to their natural environment, but caution is required since the environmental data were not acquired at the site-of-origin of the natural population. The Mehola parent occurs in the Jordan Valley with a steppic climate, and the Ashqelon parent occurs in a Mediterranean climate. Results that were found in chapter 2 were also true for the parents. The Mehola site is a drier environment and the plants had much smaller seeds, much narrower leaves with a lower leaf angle than the Ashqelon parent. Also the RGR of the Mehola parent was higher than that of the Ashqelon parent, which might imply drought avoidance strategy of the Mehola parent; growing fast and reproduce before the dry season starts.

Main conclusions

The aim of this research was to elucidate to what extent growth characteristics are genetically linked or caused by a common factor. Coincident QTLs for RGR and SLA were found on chromosome 1 (7H) and provide evidence that SLA and RGR might

be genetically linked. The overlapping QTLs of photosynthetic rate and stomatal conductance improve the physiological insight in already mapped photosynthesis-related traits. Since there are more QTLs and candidate genes found on chromosome 4 (4H) involved in photosynthesis-related traits this chromosome might be very important for area-based photosynthetic capacity in barley. However, the support intervals of most QTLs mapped, are still rather large and contain probably many genes. To reduce the interval length, fine-mapping in near-isogenic lines is required. Perhaps to begin with, a denser linkage marker map with a large number of co-dominant markers will provide a more accurate location for growth traits QTLs.

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Samenvatting

Vraagstelling

Plantensoorten hebben verschillende groeisnelheden, zelfs als ze onder identieke omstandigheden worden opgekweekt. Soorten die uit voedselrijke milieus komen, groeien bijna altijd sneller dan planten uit voedselarme milieus. Welke eigenschappen veroorzaken deze erfelijke verschillen in maximale groeisnelheid? Verschillen in de relatieve groeisnelheid (RGR; biomassa toename per eenheid van aanwezige biomassa per eenheid van tijd) van een plant worden voornamelijk veroorzaakt doordat snelle groeiers veel meer bladoppervlak per gram totale plant vormen. Twee fysiologische factoren die belangrijk zijn in het groeiproces van een plant zijn de fotosynthesesnelheid (snelheid waarmee een plant koolzuur uit de lucht haalt en omzet tot suikers die voor de groei gebruikt worden) en ademhalingssnelheid (snelheid waarmee een plant suikers weer omzet tot koolzuur waarbij energie vrijkomt). Wat echter nog niet bekend is, is waar deze eigenschappen voorkomen op de chromosomen. Zijn sommige eigenschappen misschien aan elkaar gekoppeld en erven ze daarom bijna altijd samen over of worden sommige eigenschappen bepaald door dezelfde factor? Om deze vraagstelling te beantwoorden is er gebruik gemaakt van een plantensoort; Wilde Gerst *(Hordeum spontaneum)*, de voorouder van Cultuurgerst *(Hordeum vulgare)*. Wilde Gerst heeft een grote genetische, fysiologische en morfologische variatie en komt voor in zeer verschillende milieus.

Resultaten

Voordat met de eigenlijke vraagstelling begonnen werd, is er eerst gekeken naar de variatie in groei-eigenschappen in 21 populaties van wilde gerst, die voorkomen in verschillende milieus in Israel (hoofdstuk 2). Van elke populatie werden vier accessies (= nakomelingenschap van een enkele plant verzameld in het veld) opgekweekt in een klimaatkamer onder precies dezelfde condities, met een royaal aanbod van water en nutriënten. Vervolgens werden aan deze planten een aantal fysiologische, groei- gerelateerde, chemische en morfologische groei-eigenschappen gemeten. Hieruit bleek

dat, gemiddeld over alle groei-eigenschappen, de variatie verklaard door verschillen tussen populaties en tussen accessies kleiner was dan de variatie verklaard door verschillen binnen accessies. De groei-eigenschappen waar de populaties de grootste verschillen in vertoonden waren de morfologische eigenschappen, in het bijzonder het zaadgewicht, de bladdikte en de bladbreedte. Ook werd gevonden dat zaadgewicht belangrijker is dan de relatieve groeisnelheid in het bepalen van de biomassa van planten met een leeftijd van drie weken.

In hoofdstuk 3 werd er bepaald of de variatie in groei-eigenschappen, die in hoofdstuk 2 gevonden werd, binnen een soort (Wilde Gerst) te vergelijken was met de variatie tussen 15 soorten binnen het geslacht *Hordeum*. Hieruit bleek dat de variatie binnen een soort vergelijkbaar was met de variatie tussen soorten en dat weer de grootste variatie gevonden werd in morfologische groei-eigenschappen. Ook werd er weer geconstateerd dat zaadgewicht erg belangrijk is in het bepalen van de de biomassa van planten. Er waren geen verschillen in relatieve groeisnelheid wanneer annuelle *Hordeum* soorten vergeleken werden met perenne *Hordeum* soorten. We was het zo dat annuellen over het algemeen een groter zaadgewicht hadden en daardoor ook meer biomassa.

Hoofdstuk 4 is het kernhoofdstuk van dit proefschrift en daarin wordt geprobeerd een antwoord te geven op de centrale vraagstelling, waar groei-eigenschappen voorkomen op de chromosomen en of ze aan elkaar gekoppeld zijn dan wel bepaald worden door eenzelfde factor. Om deze vraag te beantwoorden is er eerst een kruising gemaakt tussen twee populaties van Wilde Gerst die verschilden in groei-eigenschappen. Hierna werd een F_3-generatie geproduceerd door middel van zelfbestuiving van de nakomelingen. Deze werden vervolgens gebruikt om zowel een moleculaire merker-kaart te ontwikkelen (Plantenveredeling, Wageningen) als voor het karakteriseren van groei-eigenschappen. Een moleculaire merker-kaart laat zien waar de genetische verschillen zijn tussen de nakomelingen. Wanneer deze genetische verschillen gekoppeld worden aan de verschillen in kwantitatieve groei-eigenschappen in de nakomelingen dan kunnen er locaties op de chromosomen (QTL = kwantitatieve eigenschap locaties) worden aangewezen die verantwoordelijk zijn voor deze verschillen. Uit deze analyse bleek dat een QTL op chromosoom 1 verantwoordelijk is voor de variatie in relatieve groeisnelheid en dat deze samenvalt met een QTL die de variatie in de hoeveelheid bladoppervlak per gram bladgewicht bepaald. Het is moeilijk te voorspellen of deze locaties nu aan elkaar gekoppeld zijn of dat het gaat om een onderliggende factor die beide eigenschappen bepaalt. Misschien dat deze factor het bladoppervlak per gram bladgewicht bepaalt en daardoor ook de variatie in relatieve groeisnelheid. Wanneer men moet speculeren over welk gen hierbij betrokken zou zijn, zou gedacht kunnen worden aan een gen betrokken bij de activiteit van gibberellinezuur, een plantenhormoon dat doorgaans de groei van planten bevordert. Op chromosoom 4 werd een QTL

gevonden verantwoordelijk voor de variatie in fotosynthesesnelheid. Dit QTL bevond zich op dezelfde locatie als een QTL voor stomataire geleidbaarheid (= eigenschap die aangeeft hoe ver de huidmondjes geopend zijn). Waarschijnlijk is chromosoom 4 een belangrijk chromosoom in fotosynthese-gerelateerde processen. Morfologische eigenschappen zoals bladlengte, bladbreedte en de hoogte van een plant worden misschien ook wel gereguleerd door een gemeenschappelijke factor op chromosoom 2 en 6.

In hoofdstuk 5 wordt de associatie van merkers met groei-eigenschappen in 21 populaties, zoals gebruikt in hoofdstuk 2, van Wilde Gerst beschreven. Associaties van merkers met eigenschappen geven een beeld van de correlaties tussen merkers en eigenschappen. Deze analyse is minder gedetailleerd dan een QTL-analyse, omdat er geen gebruik wordt gemaakt van de informatie van merkers die 'in de buurt liggen' en daarom is deze analyse alleen gebruikt als ondersteuning van de QTL-analyse. De QTL voor relatieve groeisnelheid op chromosoom 1 werd ondersteund door een merker- eigenschap associatie. Ook de QTL voor fotosynthesesnelheid op chromosoom 4 werd ondersteund door 4 merker-eigenschap associaties, waarvan er een ook nog geassocieerd was met de hoeveelheid bladgroen per vierkante meter bladoppervlak. Er werden dus weer veel fotosynthese-gerelateerde eigenschappen op chromosoom 4 gevonden. Het bladoppervlak per gram bladgewicht verschilde niet zo veel tussen de 21 populaties en daarom werd er ook geen merker-eigenschap associatie gevonden. Op chromosoom 1, 2 en 4 werden merkers gevonden die sterk correleerden met bladdikte, bladbreedte en met zaadgewicht. Dit suggereert dat deze eigenschappen aan elkaar gekoppeld zijn.

Conclusies

Het doel van dit onderzoek was om er achter te komen in welke mate groei- eigenschappen genetisch gekoppeld zijn of gereguleerd worden door eenzelfde factor. Op chromosoom 1 werden op dezelfde locatie QTLs voor zowel relatieve groeisnelheid als de hoeveelheid bladoppervlak per gram bladgewicht gevonden. Dit suggereert dat deze eigenschappen zeer wel genetisch gekoppeld zijn. Naast QTLs voor fotosynthesesnelheid en voor stomataire geleidbaarheid werden op chromosoom 4 ook verschillende merker- eigenschap associaties voor deze eigenschappen gevonden. Dit maakt dit chromosoom een belangrijk locatie in Wilde Gerst voor fotosynthese-gerelateerde processen.

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Curriculum Vitae

Cynthia van Rijn werd op 11 mei 1972 geboren in Velden. In 1990 behaalde zij het VWO diploma aan het St. Thomas College te Venlo. In hetzelfde jaar begon zij haar studie Biologie aan de Katholieke Universiteit Nijmegen. Tijdens haar doctoraalfase voerde zij, in het kader van een hoofdvak Experimentele Plantenecologie, een onderzoek uit naar de gevoeligheid voor ethyleen in bladeren van de overstromingstolerante Moeraszuring en de overstromingsgevoelige Schapezuring. Vervolgens deed zij een hoofdvak Celbiologie van de Plant, waarbij zij een ethyleen-respons-sensor in Moeraszuring isoleerde en de regulatie van deze sensor in overstroomde bladeren van deze plantensoort bestudeerde. In december 1996 studeerde zij af en in januari 1997 begon zij als onderzoeker in opleiding bij de Projectgroep Ecofysiologie van Planten aan de Universiteit van Utrecht. Het onderzoek, waarvan de resultaten beschreven zijn in dit proefschrift, werd uitgevoerd onder leiding van Dr. Hendrik Poorter, Prof. Dr. Hans Lambers en Prof. Dr. Rens Voesenek.