Chapter 4

From individual leaf elongation to whole shoot leaf area expansion: a comparison of three *Aegilops* and two *Triticum* species

Abstract

A rapid leaf area development is a desirable trait in cereal crops. Differences between crop species or genotypes in individual leaf growth characteristics are well documented, whereas less attention has been paid to differences in the relationships between leaf growth characteristics of successive leaves and tillers. The latter is important in determining differences in leaf area expansion at the whole shoot level and whole plant growth. We investigated the relationships between several leaf characteristics and leaf position on the main stem, tiller 1 and tiller 2, for two wheat (*Triticum*) species and three wild relatives of wheat (*Aegilops* spp.). These relationships were subsequently evaluated in relation to leaf area expansion of whole shoot (RGR<sub>la</sub>), leaf photosynthetic characteristics, biomass allocation and whole plant growth (RGR<sub>dm</sub>).

In every species, leaf elongation rate (LER) was strongly and positively correlated with leaf width and sheath length, but to a lesser extent with leaf elongation duration (LED). The leaves of *Aegilops tauschii*, *Triticum aestivum* and *T. durum* elongated at twice the rate as leaves at the same positions in *Ae. caudata* and *Ae. umbellulata*. The species with the fastest-elongating leaves also had the fastest increase in LER and leaf width with leaf position, and hence had the fastest increase in leaf area. Since phyllochron and relative tillering rate did not significantly differ between the species, the faster increase in individual leaf area with leaf position resulted in faster relative leaf area expansion rates (RGR<sub>la</sub>) in *Ae. tauschii*, *T. aestivum* and *T. durum*. The high RGR<sub>la</sub> of *Ae. tauschii* in the early growth stage declined with development, to values similar to those of the other *Aegilops* species, because the increase in leaf elongation rate with leaf position slowed down considerably in this species. The high RGR<sub>dm</sub> of *Ae. tauschii*, *T. aestivum* and *T. durum* was reflected in a higher leaf area ratio (LAR) and was associated with more biomass allocated to the leaf sheaths and less to the roots. In contrast with the *Triticum* species, *Ae. tauschii* combined a high leaf area ratio (LAR) with a high rate of photosynthesis per unit leaf area, leading to a higher RGR<sub>dm</sub> in the early developmental stages of this species.
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Introduction

In areas with low rainfall, a rapid early leaf area expansion is a desirable trait in cereal crops. It leads to rapid canopy closure, reducing the evaporation from the soil surface and thus increasing crop water-use efficiency (Richards, 2000). In more favourable conditions, fast development of the canopy will make the crop more competitive with weeds for light interception (Lemerle et al., 2001). Van den Boogaard et al. (1996c) showed that in wheat growing under favourable, controlled-environment conditions, a fast leaf area expansion rate was positively correlated with total above-ground biomass and grain yield. Therefore, exploration of variation in leaf area expansion rates in different species or genotypes can give information on valuable traits to select for in wheat breeding.

In order to get a better understanding of the processes underlying variation in relative leaf area expansion rate (RGR_{la}) at the whole shoot level, data on leaf area expansion of individual, successively growing leaves and tillers are needed. The variables determining leaf area expansion of individual leaves are leaf elongation rate (LER), leaf elongation duration (LED) and leaf width. The change in these variables in subsequent leaves, together with the rate at which new leaves and tillers emerge, determine the rate of whole shoot leaf area expansion (Bultynck et al., 1999). In grasses, the initial phase of leaf growth occurs within the whorl of sheaths of the older leaves. The time the growing leaf spends inside this whorl of leaf sheaths, and hence the rate of leaf appearance from it (= inverse of the phyllochron), depends on the length of the whorl together with the LER of the growing leaf. Due to the important relationship between LER, sheath length and phyllochron, as previously found by Skinner & Nelson (1995), changes in sheath length with leaf positions are an important determinant of leaf area expansion. Expansion rates of successive leaves on the main stem of a single genotype in relation to changes in the environment are well documented (e.g., Gallagher, 1979; Gautier & Varlet-Grancher, 1996; Rodríguez et al., 1998a; Masle, 2000). Studies that include several tillers (Bos & Neuteboom, 1998a) or compare a range of genotypes or species are scarce. The first aim of the present study was to analyse inherent variation in individual leaf growth characteristics (LER, leaf width, LED, sheath length) in relation to leaf position (on main stem, tiller 1 and tiller 2) and whole shoot leaf area expansion (RGR_{la}), in five related species.

Variation in RGR_{la} may be closely associated with variation in biomass allocation and biomass production (Chapin et al., 1989, Van den Boogaard et al., 1996a). Moreover, a negative association between leaf area and the rate of photosynthesis per unit leaf area has been found in a comparison of several wheat cultivars and some of its progenitors (Evans & Dunstone, 1970, Rawson et al., 1983, Van den Boogaard et al., 1997, Villar et al., 1998), counteracting the positive effect of an increased leaf area on biomass production. The second aim of this study was to investigate the relationship between RGR_{la}, the rate of
photosynthesis per unit leaf area, the biomass allocation pattern and relative growth rate of dry mass (RGR$_{dm}$).

For this experiment we chose two wheat species (\textit{Triticum aestivum} and \textit{T. durum}) and three species from the genus \textit{Aegilops} (\textit{Aegilops umbellulata}, \textit{Ae. caudata} and \textit{Ae. tauschii}), which are wild relatives of wheat (Van Slageren, 1994). Some of the \textit{Aegilops} species have contributed in the distant past to the genome of the current bread wheat through natural hybridisation. The hybridisation between tetraploid \textit{T. turgidum} (genomic formula: AABB) and diploid \textit{Ae. tauschii} (DD) resulted in hexaploid \textit{T. aestivum} (genomic formula: AABBDD). Due to their genetic link with the wheat species and their large genetic variation, the species of the genus \textit{Aegilops} are potential donors of valuable traits for future wheat cultivars (Feldman & Sears, 1981; Damania, 1993) that may be better adapted to drier and warmer conditions, diseases and extreme temperatures. The third aim of this study was to evaluate whether the \textit{Aegilops} species have a faster leaf area expansion in their early developmental stage than some of the current wheat species/cultivars, and thus may offer genetic traits to select for and use in wheat breeding.

\section*{Materials and methods}

\subsection*{Plant material and growing conditions}

Three \textit{Aegilops} L. species and two \textit{Triticum} L. species were used: \textit{Ae. caudata}, \textit{Ae. tauschii}, \textit{Ae. umbellulata}, \textit{T. aestivum} cv. Cascades and \textit{T. durum} cv. Tamaroi. \textit{Aegilops} seeds were obtained from ICARDA (International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria) and \textit{Triticum} seeds were obtained from the Department of Agriculture Western Australia (South Perth, Australia). Prior to germination, seeds were surface-sterilised with a 2.5\% NaHClO$_3$ solution and stratified (placed on wet filter paper at 4\°C in the dark) for 7 days. Seeds were germinated on moistened filter paper in Petri dishes in a germination cabinet (day: 14 h, 50 \mu mol m$^{-2}$ s$^{-1}$ PAR, 25\°C; night: 10 h, 15\°C). After germination seedlings were transferred to trays filled with washed river-sand, saturated with de-ionised water, and placed in the growth room (day: 14 h, 420 \pm 30 \mu mol m$^{-2}$ s$^{-1}$ PAR, 23 \pm 2\°C, 70\% RH; night: 10 h, 19 \pm 2\°C, 70\% RH) for 3 days. Thereafter, seedlings were transferred (= day 0) to containers with 20 L of aerated modified Hoagland nutrient solution (2 mM NO$_3$), as described by Poorter & Remkes (1990). The pH of the nutrient solution was adjusted daily to 5.5 with H$_2$SO$_4$, and the solution was replenished weekly. Plants were rotated daily within the growth room to minimise the variation in environmental conditions for individual plants. Light competition between plants was avoided through the wide spacing of the plants in the container. As the plants grew larger, the spacing increased because some plants were harvested each week.
Destructive measurements

Eight plants per species were harvested on days 0, 7, 14, 20 (for T. aestivum and T. durum), 21 (for Ae. umbellulata, Ae. caudata and Ae. tauschii), 26 (for Ae. umbellulata, T. aestivum and T. durum) and 27 (for Ae. caudata and Ae. tauschii). The date of the last harvest differed between species depending on when leaf six on the main stem was fully elongated. Plants were separated into leaf blades, leaf sheaths and roots, and fresh weights of every portion were determined. Leaf blades were computer-scanned and analysed for leaf area, leaf length and maximum leaf width using the Win Rhizo V3.9 software (Regent Instruments, Quebec, Canada). Dry weights were determined after all plant material was dried for 48 h at 70°C.

From these data the following parameters were calculated: leaf mass ratio (LMR; leaf blade biomass per unit plant mass, g g⁻¹), stem mass ratio (SMR; leaf sheath biomass per unit plant mass, g g⁻¹), root mass ratio (RMR; root biomass per unit plant mass, g g⁻¹), leaf area ratio (LAR; leaf area per unit plant mass, m² kg⁻¹), and specific leaf area (SLA; leaf area per unit leaf mass, m² kg⁻¹). Leaf area and plant dry mass data from every harvest were ln-transformed, and relative leaf area expansion rate (RGRla, g g⁻¹ day⁻¹), relative growth rate (RGRdm, g g⁻¹ day⁻¹) and net assimilation rate (NAR; increase in total plant mass per unit leaf area per day, g m⁻² day⁻¹) were calculated for each harvest interval, using the equations of Radford (1967).

Plants reserved for the last harvest were used throughout the experiment for daily leaf growth measurements as specified below. From Villar et al. (1998) and our own preliminary experiments, we know that handling these plants daily does not affect their growth rates.

Non-destructive individual leaf growth measurements

Leaf growth measurements were conducted on eight plants per species, until leaf six on the main shoot was fully elongated. Leaf and tiller emergence were recorded daily, and leaves and tillers were identified according to Klepper et al. (1982). Phyllochron was determined for individual leaves as the time between the appearance of two successive leaf tips from the whorl of leaf sheaths. At every point of the daily non-destructive measurements the number of simultaneously growing leaves and tillers was determined. From the onset of tillering, the relative tillering rate (increase in number of tillers per number of tillers already present; tillers tiller⁻¹ day⁻¹) and the relative increase in number of simultaneously growing leaves (increase in number of simultaneously growing leaves per number of leaves already growing; leaves leaf⁻¹ day⁻¹) were calculated as the slope of the regression line through the ln-transformed number of tillers and simultaneously growing leaves versus time, respectively.

Leaf length of every growing leaf was measured daily with a ruler. Leaf elongation rate (LER, mm day⁻¹) of individual leaves (LER) was calculated as the slope of the linear regression line through the data points within the phase of linear increase in leaf length. After inspection of the data, the linear growth phase of the leaves was considered as the interval
between 20 and 80% of final leaf length for *Aegilops* species, and the interval between 10 and 90% of final length for *Triticum* species.

Leaf elongation duration (LED, days) of individual leaves was calculated as:

\[
\text{LED} = \frac{L_f}{\text{LER}}
\]

where \(L_f\) (mm) is final leaf length and \(\text{LER}\) (mm day\(^{-1}\)) is leaf elongation rate.

**Gas exchange measurements**

At the last harvest date, gas exchange was measured on the youngest fully expanded leaf on the main stem of three plants per species. Gas exchange measurements were carried out with a LiCor 6400 system using the red and blue LED light source (LiCor, Lincoln, NE, USA). First, the rate of CO\(_2\) assimilation was measured at ambient CO\(_2\) concentration, at a saturating light intensity (2000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); \(A_{\text{max}}\)) and then at a light intensity similar to that in the growth room (430 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); \(A_{\lambda}\)). Thereafter, the response to intercellular CO\(_2\) was measured at a light intensity of 2000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) and at external CO\(_2\) concentrations declining from 1200 \(\mu\)mol mol\(^{-1}\) to 50 \(\mu\)mol mol\(^{-1}\).

It was assumed that, at low intercellular CO\(_2\) partial pressures, the assimilation of CO\(_2\) was limited solely by the amount, activity and kinetic properties of Rubisco (Wullschleger, 1993). At these low intercellular CO\(_2\) partial pressure values, Rubisco activity (\(V_c\)) was obtained by fitting the following equation to the rates of CO\(_2\) assimilation (Von Caemmerer & Farquhar, 1981):

\[
A = \frac{V_c (C - \Gamma_*)}{(C + K_m)} - R
\]

where \(C\) is the intercellular CO\(_2\) partial pressure (assumed here to be equal to that at the site of carboxylation), \(\Gamma_*\) is the CO\(_2\) compensation point in the absence of dark respiration (\(R\)), and \(K_m\) is the effective Michaelis-Menten constant for CO\(_2\). The kinetic constants for Rubisco were assumed to be equal to those determined for tobacco (Von Caemmerer *et al.*, 1994), namely 3.69 Pa for \(\Gamma_*\) and 73 Pa for \(K_m\) at 25°C. As our data were obtained at growth temperature (23°C), parameter values were calculated using the Arrhenius equation and activation energies given by De Pury & Farquhar (1997). At high intercellular CO\(_2\) partial pressures, it was assumed that CO\(_2\) assimilation was limited by the electron transport activity (\(J\)). \(J\) was then obtained by fitting the following equation through the rates of CO\(_2\) assimilation at high intercellular CO\(_2\) partial pressures (Von Caemmerer & Farquhar, 1981):

\[
A = \frac{J(C - \Gamma_*)}{(4C + 8\Gamma_*)} - R
\]

Total nitrogen concentration of the leaves on which photosynthesis was measured, was determined with an automatic C-H-N analyser (Leco CHN 1000, St. Joseph, MI, USA).
Statistical analysis

Data were analysed with SPSS 8.0 for Windows statistical software (SPSS, Inc., Chicago, IL, USA). For each leaf position, differences in LER, leaf width and LED between species were analysed by one-way ANOVA ($\alpha = 0.05$). The results from this analysis were used to calculate the LSD. Relations between leaf parameters were tested with linear regression equations. Differences between species in biomass allocation parameters were analysed by two-way ANOVA with species and time as factors. Differences amongst species in $\text{RGR}_{\text{dm}}$ and $\text{RGR}_{\text{la}}$ were tested by two-way ANOVA of the ln-transformed plant dry mass and leaf area data with species and time as the independent factors (Poorter & Lewis, 1986). A significant interaction between species and time indicates a difference in $\text{RGR}_{\text{dm}}$ or $\text{RGR}_{\text{la}}$. Differences amongst species in gas exchange parameters, relative tillering rate and relative increase in simultaneously growing leaves were analysed with a one-way ANOVA, followed by a Tukey post hoc test at $\alpha = 0.05$.

Results

LER, LED and leaf width of individual leaves

The individual leaves on the main stem, tiller 1 and tiller 2 of *Ae. tauschii*, *T. aestivum* and *T. durum* had a larger leaf area than the ones at similar leaf positions of *Ae. caudata* and *Ae. umbellulata* (Fig. 1A), due to a faster leaf elongation and wider leaves (Fig. 1B and C). No such distinct differences were found in LED between the species (Fig. 1D). Figure 2 shows the relationship between LER and leaf width or LED for all the leaves presented in Figure 1. LER was positively correlated with leaf width within every species ($p<0.001$, $r^2$ between 0.171 for *Ae. umbellulata* and 0.628 for *T. aestivum*), and the correlation became stronger when all the species were grouped together ($p<0.001$, $r^2=0.71$) (Fig. 2A). The relationship between LER and LED was much less clear, both within and amongst species ($p<0.001$, $r^2=0.049$) (Fig. 2B). In general, irrespective of the species, faster-growing leaves were wider and grew for a slightly longer time and, consequently, had a larger leaf area than slower-growing leaves.

LER, LED and leaf width increased with increasing leaf number on a tiller, in most species (Fig. 1). The increase in LED did not differ significantly amongst most species whereas the increase in LER and leaf width was significantly faster in the two *Triticum* species and *Ae. tauschii* compared with the slower-elongating *Ae. umbellulata* and *Ae. caudata* (Fig. 1). In the latter two species leaf width remained constant in successive leaves. In the fast-elongating *Ae. tauschii* and the two *Triticum* species, LER and leaf width increased by more than 150% from the first to the sixth leaf on the main stem. In the slow-elongating *Ae. umbellulata* and *Ae. caudata*, LER increased by approximately 100% from
Figure 1. Leaf area (A), leaf elongation rate (B), leaf width (C), and leaf elongation duration (D) of successive leaves on main stem, tiller 1 and tiller 2, of *Ae. umbellulata*, *Ae. caudata*, *Ae. tauschii*, *T. aestivum* and *T. durum*. Symbols denote means of 8 plants per species. Vertical bars represent LSD.
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the first to the sixth leaf on the main stem, and leaf width increased by approx. 20%. On tiller 1 and tiller 2, *Ae. tauschii* and the two *Triticum* species showed a higher relative increase in leaf width from the first to the third leaf than *Ae. umbellulata* and *Ae. caudata*. However, the species no longer differed in their relative increase in LER. The relative increase in LED was similar amongst all the species on main stem, tiller 1 and tiller 2, and was generally less than 50%.

![Figure 2. Relationship between (A) leaf elongation rate and leaf width, and between (B) leaf elongation rate and leaf elongation duration, of individual leaves of *Ae. umbellulata*, *Ae. caudata*, *Ae. tauschii*, *T. aestivum* and *T. durum*. Symbols denote mean values (±SE) of 8 leaves per leaf position. The lines indicate the significant linear regressions, derived from the individual values of each leaf.](image)

**Leaf appearance rate: leaf and tiller emergence**

There were no significant differences in phyllochron (=inverse of leaf emergence) between the different tillers and species (Table 1; two-way ANOVA with tiller and species as factors and \( \alpha = 0.05 \), data not shown). The timing of leaf emergence depends on the LER of the growing leaf and sheath length of the previously growing leaf (Miglietta, 1991; Skinner & Nelson, 1995). Figure 3 shows that both LER of a specific leaf (leaf n) and sheath length of the preceding leaf (leaf n-1) were greater in *Ae. tauschii* and the two *Triticum* species.
Table 1. Comparison amongst species of phyllochron (days), relative tillering rate (tillers tiller\(^{-1}\) day\(^{-1}\)), relative increase in number of simultaneously elongating leaves (leaves leaf\(^{-1}\) day\(^{-1}\)). Means with different letters are significantly different (post hoc Tukey; p<.005).

<table>
<thead>
<tr>
<th></th>
<th>Phyllochron</th>
<th>Relative tillering rate</th>
<th>Relative increase in simultaneously growing leaves</th>
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<tbody>
<tr>
<td></td>
<td>Main stem</td>
<td>Tiller 1</td>
<td>Tiller 2</td>
</tr>
<tr>
<td><em>Ae. umbellulata</em></td>
<td>4.2</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td><em>Ae. caudata</em></td>
<td>4.7</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td><em>Ae. tauschii</em></td>
<td>4.5</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td><em>T. aestivum</em></td>
<td>4.2</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td><em>T. durum</em></td>
<td>3.9</td>
<td>4.0</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Although *Ae. umbellulata* started to produce tillers earlier (day 6) than the other species (between day 8 and day 10), in all species the first tiller emerged when the third and fourth leaf on the main stem were growing and the tillers emerged in the same sequence (data not shown). From the onset of tillering, no significant difference in relative tillering rate was found between most of the species (Table 1): the two *Triticum* species had a higher relative tillering rate (>0.060 tillers tiller\(^{-1}\) day\(^{-1}\)) than the *Aegilops* species (<0.055 tillers tiller\(^{-1}\) day\(^{-1}\)), but the difference was only significant for *T. aestivum* (0.063 tillers tiller\(^{-1}\) day\(^{-1}\)) (Table 1). The same differences amongst species were observed for the relative increase in

Figure 3.
Relationship between leaf elongation rate of leaf \(n\) and sheath length of leaf \(n-1\) of individual leaves presented in Figure 1 of *Ae. umbellulata*, *Ae. caudata*, *Ae. tauschii*, *T. aestivum* and *T. durum*. Symbols denote mean values (±SE) of 8 leaves per leaf position. The line indicates the significant linear regression, derived from the individual values of each leaf.
Figure 4. Ontogenetic changes in (A) relative growth rate of plant dry mass (RGRdm), (B) leaf area ratio (LAR), (C) net assimilation rate (NAR), and (D) relative leaf area expansion rate (RGRla), of *Ae. umbellulata*, *Ae. caudata*, *Ae. tauschii*, *T. aestivum* and *T. durum*. Arrows in (D) indicate onset of tillering for each species. Error bars in (B) indicate standard error (n=8). The error bars of RGR were omitted for clarity’s sake and for NAR it was not possible to calculate standard error. See Results for statistical evaluation of these parameters.

Simultaneously elongating leaves, calculated from the start of tillering, but none of these differences were significant (Table 1). Before the onset of tillering, the number of simultaneously growing leaves on the main stem was constant and similar for all species: it fluctuated between 1 and 2 leaves growing simultaneously (data not shown).

**Whole plant growth and biomass allocation**

Figures 4 and 5 present biomass allocation, LAR, SLA, NAR, and relative growth rate of dry mass (RGRdm) and leaf area (RGRla) as a function of plant size. Two-way analysis of variance, with species and time as fixed factors, showed highly significant (p<0.001) main effects and interactions for all the parameters presented in figures 4 and 5. At the start of the growing period, the relative growth rate (RGRdm) was highest in *Ae. tauschii*, lowest in *Ae. umbellulata* and *Ae. caudata*, and intermediate in *T. aestivum* and *T. durum* (Fig. 4A). The faster RGRdm of *Ae. tauschii* was associated with a higher LAR, whereas the intermediate...
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Figure 5. Ontogenetic changes in (A) specific leaf area (SLA), (B) leaf mass ratio (LMR), (C) stem mass ratio (SMR), and (D) root mass ratio (RMR), of *Ae. umbellulata*, *Ae. caudata*, *Ae. tauschii*, *T. aestivum* and *T. durum*. Error bars indicate standard error (n=8). See Results for statistical evaluation of the parameters.

RGR$_{dm}$ of the *Triticum* species was associated with a higher NAR compared with *Ae. umbellulata* and *Ae. caudata* (Figs 4B and C). In *Ae. tauschii*, RGR$_{dm}$ and LAR decreased by approx. 60% over the experimental period and reached values similar to those of the other *Aegilops* species (Figs 4A and B). The two *Triticum* species also decreased their RGR$_{dm}$ initially due to a decrease in NAR. However, the NAR increased again at the end of the experimental period along with a strong increase in LAR, resulting in a faster RGR$_{dm}$ than in the *Aegilops* species (Figs 4A, B and C). The change in RGR$_{dm}$ with ontogeny showed a similar pattern as that of RGR$_{dm}$. RGR$_{dm}$ was faster in the two *Triticum* species and *Ae. tauschii* at the start of the growing period, and in the *Aegilops* species it decreased more with increasing plant size (Fig. 4D).

LAR in the *Aegilops* species decreased as a result of the decreasing SLA (Fig. 5A), whereas LAR in the *Triticum* species increased due to a stronger increase in LMR and a lesser decrease in SLA (Fig. 5B). At the start of the experimental period, the biomass allocation pattern of *Ae. tauschii* resembled that of the other *Aegilops* species: they allocated more to the leaf blades (high LMR) and leaf sheaths (high SMR) and less to the roots (low

45
than the *Triticum* species did (Figs 5B, C and D). The higher SMR in the *Aegilops* species at the first harvest probably resulted from a relatively longer coleoptile, which was included in the stem fraction. At a later stage of their development, the biomass allocation pattern of *Ae. tauschii* resembled that of the two *Triticum* species: LMR and SMR increased with development while RMR deceased (Figs 5B, C and D).

**Photosynthetic characteristics**

Table 2 shows the photosynthetic characteristics for the last fully expanded main stem leaf in all the species at the last harvest date. The *Aegilops* species had faster rates of photosynthesis per unit leaf area than the *Triticum* species, both at a PPFD of 430 μmol m⁻² s⁻¹ (*A*₄₃₀), the light level at which the plants were grown, and at saturating light levels (*A*ₘₐₓ). The faster rates of photosynthesis per unit leaf area in *Aegilops* were associated with a higher nitrogen concentration, a higher Rubisco activity (*V*ₖ) and a higher electron transport activity (*J*) per unit leaf area than in *Triticum*. Per unit nitrogen, the rate of photosynthesis (*A/N*), Rubisco activity (*V*ₖ/N) and electron transport activity (*J/N*) did not differ between the *Triticum* and *Aegilops* species, suggesting that similar proportions of leaf nitrogen were allocated to Rubisco and electron transport. *Ae. tauschii*, the *Aegilops* species with the faster-elongating leaves, tended to have a lower leaf nitrogen concentration but a similar rate of photosynthesis per unit leaf area than the *Aegilops* species with the slow-elongating leaves.

Table 2. Photosynthetic parameters of the youngest fully expanded main stem leaf of three *Aegilops* and two *Triticum* species, at the last harvest date. Values of leaf nitrogen concentration per unit leaf area (leaf N), rate of photosynthesis at 430 μmol m⁻² s⁻¹ per unit leaf area (*A*₄₃₀), rate of photosynthesis at 2000 μmol m⁻² s⁻¹ per unit leaf area (*A*ₘₐₓ) and per unit leaf nitrogen (*A*ₘₐₓ/N), Rubisco activity at 2000 μmol m⁻² s⁻¹ per unit leaf area (*V*ₖ) and per unit leaf nitrogen (*V*ₖ/N), and electron transport activity at 2000 μmol m⁻² s⁻¹ per unit leaf area (*J*) and per unit leaf nitrogen (*J/N*). Different letters indicate significant differences between species (n=3).

<table>
<thead>
<tr>
<th></th>
<th><em>Ae. umbellulata</em></th>
<th><em>Ae. caudata</em></th>
<th><em>Ae. tauschii</em></th>
<th><em>T. aestivum</em></th>
<th><em>T. durum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf N (μmol m⁻²)</td>
<td>173ab</td>
<td>189a</td>
<td>153bc</td>
<td>127cd</td>
<td>121d</td>
</tr>
<tr>
<td><em>A</em>₄₃₀ (μmol m⁻² s⁻¹)</td>
<td>22.6a</td>
<td>20.1ab</td>
<td>21.9a</td>
<td>16.7bc</td>
<td>14.9c</td>
</tr>
<tr>
<td><em>A</em>ₘₐₓ (μmol m⁻² s⁻¹)</td>
<td>40.0*</td>
<td>34.6b</td>
<td>35.0a</td>
<td>27.6bc</td>
<td>25.9c</td>
</tr>
<tr>
<td><em>V</em>ₖ (μmol m⁻² s⁻¹)</td>
<td>174a</td>
<td>167a</td>
<td>159a</td>
<td>119b</td>
<td>120b</td>
</tr>
<tr>
<td><em>J</em> (μmol m⁻² s⁻¹)</td>
<td>285*</td>
<td>237ab</td>
<td>246a</td>
<td>187bc</td>
<td>176c</td>
</tr>
<tr>
<td><em>A</em>ₘₐₓ/N (μmol mol⁻¹ s⁻¹)</td>
<td>234*</td>
<td>176b</td>
<td>222ab</td>
<td>215bc</td>
<td>209bc</td>
</tr>
<tr>
<td><em>V</em>ₖ/N (μmol mol⁻¹ s⁻¹)</td>
<td>1.01*</td>
<td>0.87a</td>
<td>1.04a</td>
<td>0.94a</td>
<td>0.99a</td>
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<tr>
<td><em>J/N</em> (μmol mol⁻¹ s⁻¹)</td>
<td>1.66*</td>
<td>1.26b</td>
<td>1.60ab</td>
<td>1.48ab</td>
<td>1.45ab</td>
</tr>
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Discussion

Relationship between LER, LED, leaf width and leaf position

In all the species in this study, leaf area increased with leaf position on the main stem, tiller 1 and tiller 2. This increase was strongly associated with an increase in leaf elongation rate (LER) and leaf width, but much less with an increase in duration of leaf elongation (LED). Although increases in leaf length and leaf width with leaf position have been shown before in cereal crops (e.g. Williams & Rijven, 1965; Gallagher, 1979; Bos & Neuteboom, 1998a), comparisons amongst species are scarce. Leaf width and LER increased faster with leaf position in the species with the widest and longest leaves (Ae. tauschii, T. aestivum, T. durum) compared with the species with the smaller leaves (Ae. caudata, Ae. umbellulata), where an increase in LER and leaf width was often lacking. Contrary to the increase in LER and leaf width with leaf number, the increase in LED was not significantly different amongst the species in the present study and therefore did not contribute to differences in leaf area amongst these species.

In a previous paper, we have shown that the twofold difference in LER and leaf width of the third leaf on the main stem between Ae. caudata and Ae. tauschii was associated with a twofold difference in length and width of the leaf meristem (i.e. the number of dividing cells in length and width) (Chapter 2). Beemster et al. (1996) have also shown that the increase in LER in three successive leaves of wheat is associated with an increase in the length of the leaf meristem. Possibly, the faster increase in length and width with leaf position in the fast-elongating species in the present study resulted from a faster increase in leaf meristem size of successive leaves. In several cases, increases in LER and leaf width with leaf position have been associated with increases in apical dome size (Abbe et al., 1941; Kirby, 1974; Pieters & Van den Noort, 1988; Bos & Neuteboom, 1998a). However, some evidence suggests that LER and leaf width are controlled independently and that leaf width is not controlled by the apical dome size. The independent control of LER and leaf width is suggested by our own data as well as those of other authors. Our results show that, at least in some of the species or in some of the tillers, the relative increase in LER with leaf position differed from that in leaf width. Moreover, the AFLP-markers that correlate with LER differ from the ones that are correlated with leaf width, in 46 Ae. tauschii accessions (M.W. ter Steeg, personal communication). Beemster et al. (1996) provide additional evidence for leaves of wheat seedlings grown at different soil resistances. The growing leaves in their study differed in sensitivity of the number of formative divisions (determines number of parallel cell files and is related to leaf width) and of the number of proliferative divisions (determines number of cells along a cell file and is related to LER) to the treatment. Beemster & Masle (1996) also showed that the reduction in leaf width that was induced by
the treatment was not related to a reduction in apical dome size, but to changes in cellular processes that take place after leaf initiation.

**Regulation of leaf and tiller appearance**

The relationship between LER of a growing grass leaf and sheath length of the preceding leaf determines the time needed for the growing leaf to appear from the whorl of sheaths, and hence the phyllochron (Skinner & Nelson, 1995). The fast-growing species in our study had both longer leaf sheaths and faster LER, resulting in similar phyllochron values for all species. Within each species, however, sheath length increased faster than LER increased whereas phyllochron remained constant for successive leaves (Fig. 3). Since the interval between initiations of successive leaves seems to be constant under constant environmental conditions (Hay & Kemp, 1990, Rodríguez et al., 1998b), the most likely explanation is that the initiation of leaf elongation was progressively earlier in successive leaves. Skinner & Nelson (1995) showed that initiation of leaf elongation in the youngest leaf primordium of tall fescue was synchronized with ligule initiation in the second youngest leaf, cessation of cell division in the sheath of the third oldest leaf, and initiation of tiller elongation at the axillary bud associated with that leaf. This observation shows a close association between the development of successive leaves and their associated tillers, as well as between the timing of leaf and tiller appearance. That may explain why we found very little variation in both phyllochron and relative tillering rate between the species in this study. Although the different species had similar relative tillering rates and the first tiller emerged at the same stage of development, the species differed in the time at which they reached this stage of development. Especially *Ae. umbellulata* reached the four-leaves stage at which tillering commences earlier than the others. This was either due to a difference in the timing of germination or in the rate of shoot development. We have no data on the exact timing of germination, but the phyllochron gives an approximation of the developmental rate (McMaster, 1997). The phyllochron tended to be shorter in *Ae. umbellulata* compared with the other *Aegilops* species, but was similar to that of the *Triticum* species. Therefore, differences in the timing of germination also played a role.

**Whole shoot leaf area expansion**

Differences in individual leaf growth affected the relative leaf area expansion rate (RGRₗₐ), mainly during the very early growth stages. The faster increase in leaf width and leaf length with leaf position on the main stem of the fast-expanding *Ae. tauschii, T. durum* and *T. aestivum* resulted in a faster RGRₗₐ during early growth compared with the slow-expanding *Ae. umbellulata* and *Ae. caudata. As the increase in leaf elongation rate with leaf position slowed down, RGRₗₐ quickly declined in all species, which clearly showed that a linear increase in leaf area cannot lead to exponential growth without tillering (Van Loo, 1992;
In accordance with other studies on wheat (Longnecker et al., 1993; Rodríguez et al., 1998b; Miralles & Richards, 2000), the first tiller started to appear on all the species in this study when the third and fourth leaf on the main stem were growing. Tillering reduced the rate of decline in RGR_{la} in Triticum but not in Aegilops. This was partly due to the slower increase in LER and leaf width with leaf position on the newly formed tillers of the Aegilops species compared with the Triticum species. The slightly higher tillering rates of both Triticum species probably also played a role in this.

We have focused in this experiment on the RGR_{la}. However, the absolute size of leaf area during early growth is also important in determining a high ground cover, final biomass and yield (López-Castañeda et al., 1996). The species in the present study that had a higher RGR_{la} (Ae. tauschii, T. aestivum and T. durum) also achieved a higher total leaf area early in development. Since total leaf area increased slower in Ae. tauschii than in the two Triticum species, the advantage of a large leaf area in Ae. tauschii disappeared as plants grew older and larger. Several studies have shown that seed or embryo size is more important than RGR in determining plant and leaf size of seedlings (e.g. Chapin et al., 1989; López-Castañeda et al., 1996, Van Rijn et al., 2000). We did not measure seed size in this experiment but, assuming leaf width of the first seedling leaf is a good measure of embryo size (López-Castañeda et al., 1996), this would suggest that the Triticum species had larger embryos than the Aegilops species (Fig. 1C). Despite its supposedly small embryo, Ae. tauschii achieved a larger total leaf area than the other Aegilops species early in development due to a faster RGR_{la}, showing that a high RGR_{la} can be important during early development.

Whole plant growth and biomass allocation

The faster increase in leaf area expansion with leaf position in Ae. tauschii, T. aestivum and T. durum, compared with Ae. umbellulata and Ae. caudata, was reflected in the higher leaf area ratio (LAR) in these species. Initially, the Triticum species had a low LAR, even lower than that of the slow-expanding Ae. umbellulata and Ae. caudata, because they invested proportionally more carbon in their roots (high RMR). However, upon elongation of the first few leaves of the Triticum species, the RMR quickly dropped below that of the Aegilops species, whereas the LAR increased to values considerably above those of the Aegilops species. It is likely that the high demand for carbon in the division and expansion zones of the growing leaves (Hu et al., 2000, Schäufele & Schnyder, 2001) resulted in more carbon being used in the shoot instead of going to the roots. The relatively high RMR for Aegilops compared with Triticum has been found before (Van den Boogaard & Villar, 1998) and may be an adaptation to growth in dry and nutrient-scarce environments (Villar et al., 1998). In contrast with the Triticum species, Ae. tauschii had a high LAR at the start of the growing period, which quickly dropped below that of the Triticum species due to a decrease in SLA with development. SLA in the Triticum species however, remained rather constant over the
growing period. Species occurring in dry environments, like the *Aegilops* species, probably benefit from making thicker leaves (lower SLA) (Lambers & Poorter, 1992).

In the early developmental stage, *Ae. tauschii* was able to combine a high LAR with a relatively high NAR, and this led to a considerably higher biomass production (high RGRdm). This growth advantage disappeared as the plants grew larger and LAR dropped below that of the *Triticum* species. At the end of the experimental period, the higher LAR of the *Triticum* species was associated with a lower NAR and lower rates of photosynthesis per unit leaf area than in the *Aegilops* species. A negative association between leaf area and the rate of photosynthesis per unit leaf area has been found by several authors in wheat cultivars as well as wheat ancestors (Evans & Dunstone, 1970, Rawson et al., 1987: individual leaf area, Van den Boogaard et al., 1997: leaf area ratio, Villar et al., 1998: total leaf area). In the present study, most of the differences in photosynthetic rate per unit leaf area were explained by the lower leaf nitrogen concentration per unit leaf area of the *Triticum* species, as the photosynthetic rate expressed per unit leaf nitrogen was similar to that of the *Aegilops* species. Similarly, Evans (1985) showed that variation in photosynthetic rate per unit leaf area amongst three wheat species and one *Aegilops* species was strongly related to variation in leaf nitrogen concentration per unit leaf area. The lower leaf nitrogen concentration of the species in the present study was a consequence of both the higher SLA and the lower leaf nitrogen concentration per unit mass.

The fast-growing *Ae. tauschii, T. aestivum* and *T. durum* have a higher SMR than the slow-growing species in this study, a relationship that was also found by Van den Boogaard & Villar (1998) in a comparison of 22 *Aegilops* species with 10 *Triticum* cultivars. These authors suggested that gibberellins might be involved, as gibberellins have previously been associated with differences in RGR and SMR (Nagel et al., 2001a). Our results support such a hypothesis, since we have shown that the species with the highest SMR also have the fastest-elongating leaves, while several authors reported on the important stimulating effect of gibberellins on leaf elongation rate (Toukinston et al., 1995; Chandler & Robertson, 1999). In a forthcoming paper (Chapter 5), we explore the influence of GA on leaf expansion and biomass allocation in these *Aegilops* species.

**Future use of Aegilops?**

From the *Aegilops* species used in the present study, only *Ae. tauschii* was able to reach the same high RGRdm as the *Triticum* species. Moreover, in the early growth stages *Ae. tauschii* had an even higher LAR than the *Triticum* species. Since the development of a large leaf area has been shown to be related to high yield in several cereal crops (López-Castañeda et al., 1996), it is worthwhile to further explore this trait in *Ae. tauschii*, the wild relative from which wheat inherited the D-genome (Feldman & Sears, 1981). In further studies, more accessions of *Ae. tauschii* need to be investigated under field conditions. From this study, we
can also conclude that there is a large variation in leaf growth characteristics within the *Aegilops* genus, which makes this genus ideal for future experiments on the regulation of leaf growth.

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