Epidermal cell division and cell elongation in two *Aegilops* species with contrasting leaf elongation rates

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Abstract. The 2-fold difference in final length of leaf number three on the main stem between the fast-growing *Aegilops tauschii* L. and the slow-growing *Aegilops caudata* L. is correlated with a difference in leaf elongation rate (LER), and not in duration of leaf elongation. In this paper the cellular basis of inherent differences in LER between these species was investigated.

The dynamics of abaxial epidermal cells along the growth zone of leaf number three on the main stem of both species was analysed by means of a kinematic analysis. The faster LER of *Ae. tauschii* compared with that of *Ae. caudata* was associated with (i) a larger leaf basal meristem and cell elongation-only zone, and (ii) a faster cell production rate owing to a larger number of dividing cells. Cell division rate, mature cell size and cell elongation rate did not differ between the two species. The lack of variation in cell expansion rate between the species was supported by a similar capacity of both species to extend their isolated cell walls upon acidification.

These data suggest that differences in the number of dividing cells can bring about differences in the number of simultaneously elongating cells, and hence in LER.

**Keywords**: *Aegilops*, cell division, cell expansion, cell-wall extensibility, kinematic analysis, leaf basal meristem size, leaf elongation rate, strain rate.

Introduction

Fast expansion of the first few leaves of cereal crops has been shown to benefit yield (Whan et al. 1991; López-Castañeda and Richards 1994). Rapid, early expansion of leaf area leads to rapid canopy closure, and thereby reduces evaporation from the soil surface, thus, increasing water availability for the plant (Richards et al. 1993). It also makes the crop species more competitive with weeds for light interception (Lemerle et al. 2001). Several authors have studied the effects of environmental changes on the cellular processes underlying leaf expansion rates in crop species (MacAdam et al. 1989; Ben-Haj-Salah and Tardieu 1995; Beemster et al. 1996; Fricke et al. 1997). Fewer studies have compared the cellular basis of intra- and interspecific differences in leaf expansion (Volenc and Nelson 1981; Fiorani et al. 2000; Masle 2000).

In monocotyledonous species, growing leaves expand predominantly in length. The rate of leaf elongation is determined by the number of elongating cells, and the rate and duration of cell expansion. In leaves, epidermal cell production and cell elongation occur in the growth zone, which is located at the base of the leaf and enclosed by the sheaths of older leaves (Volenc and Nelson 1981; MacAdam et al. 1989; Schnyder et al. 1990). In the most basal part of the growth zone, *i.e.* the leaf basal meristem, cells are produced in parallel cell files and displaced away from the leaf base as a result of continuous production and elongation of new cells within the same file. As the cells are being displaced through the leaf basal meristem, they continue to divide and elongate until they reach the elongation-only zone. There, cells stop dividing and continue to elongate until they reach their mature size. These

Abbreviations used: $L_{\text{div}}$, length of cell division zone; LER, leaf elongation rate; $L_{\text{ez}}$, length of elongation-only zone; $L_{\text{gz}}$, length of growth zone; $N_{\text{div}}$, number of cells per file in the division zone; $N_{\text{ez}}$, number of cells per file in elongation-only zone; $N_{\text{gz}}$, number of cells per file in the growth zone; PAR, photosynthetically active radiation; $T_{\text{div}}$, residence time in cell division zone; $T_{\text{ez}}$, residence time in elongation-only zone.
processes result in a typical distribution of cell lengths with distance from the leaf base. Schnyder et al. (1990) have shown that this cell-length profile is constant during the period of linear growth in *Lolium perenne*. It is assumed that this is also true for other grasses. When leaf elongation is in steady-state, the derivative of the cell-length distribution along the growth zone can be used to determine the distribution of relative cell elongation rates, i.e. strain rates, along the elongation-only zone (Silk et al. 1989). In a comparison of four *Poa* species, Fiorani et al. (2000) found that interspecific variation in LER was strongly correlated with variation in cell production rate, but not with that in maximum cell elongation rate and cell elongation duration. Similar interspecific comparisons of leaf cellular processes are lacking. Therefore, we examined the relationships between LER and its underlying cellular processes for *Aegilops* species, which are related to the agronomically important *Triticum* species. *Aegilops* species are becoming increasingly important as potential sources of valuable traits that can be used in wheat breeding (Damania 1993).

Differences in leaf-cell elongation rates are more often correlated with differences in cell-wall extensibility than with differences in cell turgor (Van Volkenburgh 1999, and references therein). A positive correlation has been shown between leaf growth rate and the capacity of cell walls to expand upon acidification, in dicotyledonous as well as monocotyledonous leaves (Van Volkenburgh and Boyer 1985; Van Volkenburgh et al. 1985a). Several studies have shown that plant cells in growing tissues are able to acidify the apoplast, thereby activating cell-wall-loosening proteins, called expansins (Cosgrove 2000, and references therein). It is possible that species differ in responsiveness of the apoplast to acidification, resulting in a difference in cell elongation rate and hence leaf elongation rate.

The first aim of this study was to determine which cellular growth processes determine the inherent differences in LER between two *Aegilops* species. Cell division parameters, cell-length distribution and strain-rate distribution along the growth zone of leaf number three on the main stem were examined. Second, the responsiveness to acidification of isolated cell walls from the growing zone of leaves of these two species was determined.

**Materials and methods**

**Plant material and growing conditions**

Seeds of *Aegilops caudata* L. and *Aegilops tauschii* L. were obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA, Aleppo, Syria). Prior to germination, seeds were surface-sterilised with a 2.5% (v/v) NaClO solution and stratified (placed on wet filter paper at 4°C in the dark) for 7 d. Seeds were germinated on moistened filter paper in petri dishes, in a germination cabinet [day: 14 h, 50 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR), 25°C; night: 10 h, 15°C]. After germination, seedlings were transferred to trays with washed river-sand, saturated with de-ionised water, and placed in a growth room [day: 14 h photoperiod, 445 ± 15 μmol m⁻² s⁻¹ PAR, 23 ± 2°C, 70% relative humidity (RH); night: 10 h, 19 ± 2°C, 70% RH]. After 3 d the seedlings were transferred to containers with 20 L of the following aerated nutrient solution: 795 μM KNO₃, 603 μM Ca(NO₃)₂, 270 μM MgSO₄, 190 μM KH₂PO₄, 40 μM Fe-EDTA, 20 μM H₃BO₃, 2 μM MnSO₄, 0.85 μM ZnSO₄, 0.25 μM Na₂MoO₄ and 0.15 μM CuSO₄. The pH of the nutrient solution was adjusted daily to 5.5 with H₂SO₄ and the solution was replenished weekly. Plants were rotated daily within the growth room to minimise the variation in environmental conditions for individual plants.

**Individual leaf growth measurements**

Individual leaf-growth measurements were conducted on leaf number three of the main shoot for both species, on a first set of eight plants per species. Leaf length, from the leaf tip to the level from which leaves emerge from the hydroporons container, was measured daily (always 1 h after the onset of the light period) with a ruler, from the day they emerged from the sheath of leaf number two until they were fully elongated. LER (mm d⁻¹) of individual leaves was calculated as the slope of the linear regression line through the data points within the period of linear increase in leaf length. The linear growth phase of the leaves was determined as the interval between 20 and 80% of final leaf length; outside this interval the increase in leaf length with time was not linear. Leaf elongation duration (in days) of individual leaves was calculated as the ratio between final leaf length and LER of those individual leaves.

**Cell length measurements**

A second set of five plants per species was used for determining epidermal-cell-length profiles of leaf number three on the main shoot. The leaves were harvested within the first 2 d after emergence from the sheath of leaf number two, when they were in the linear growth phase. Leaves were cut at the base of the basal meristem, transferred immediately to boiling methanol for chlorophyll removal, and subsequently transferred to 90% lactic acid for clearing and storage. Leaf length was measured before and after boiling in methanol and no tissue shrinkage was observed.

The cleared leaves were mounted on a light microscope (Olympus BX60 F5, Tokyo, Japan), which was connected to a Panasonic CCD camera (model GP-KR222E). Two epidermal cell files, adjacent to stomatal cell files, on the abaxial side of every leaf were selected. Cell lengths of all the cells along those files were measured from video images (total magnification 100x), using Video Trace image-measurement software (Leading Edge, Marion, SA, Australia). For each cell file, a cell-length profile along the leaf axis was obtained by plotting the length of each cell against its distance from the leaf base. Data from the two cell files per leaf were combined, and then smoothed and interpolated using the procedure described by Beemster and Baskin (1998). Mature cell length and the length of the growth zone (L₀) were determined from the smoothed data from each leaf. Mature cell length was estimated as the average cell length of all data points distal to the position where the change in cell length between successive data points was less than or equal to zero. The length of the zone was estimated as the distance from the leaf base to the position where cells reached 95% of mature cell length. These values were averaged among leaves and used for subsequent calculations.

**Estimation of leaf basal meristem length**

A third set of five plants per species was used to estimate the length of the leaf basal meristem in leaf number three on the main stem. The leaves were harvested within the first 2 d after emergence from the sheath of leaf number two, when they were in the linear growth phase. The leaves were cut at the base of the basal meristem and transferred immediately to a 3:1 (v/v) solution of absolute ethanol:glacial acetic
acid at 4°C for at least 24 h, for tissue fixation and chlorophyll removal. Subsequently, the cleared leaves were hydrolysed in 3 M HCl for 20 min and immersed for at least 2 h in a Feulgen dye prepared with basic fuchsin (rosanilin; Merck, Kilsyth, Vic., Australia), for staining the nuclei, according to the method of Moses et al. (1997).

The stained leaves were transferred to a microscope slide, immersed in a droplet of 0.1 M Na2S2O4 dissolved in 0.15 M HCl and covered with a coverslip. The same image analysis set-up was used for estimating the length of the leaf basal meristem as for the cell-length measurements (total magnification 200×). Ten epidermal cell files next to stomatal cell files were selected on the abaxial side of the leaf. The distance between the most distal mitotic figure in a selected cell file, and the base of the leaf was measured. This was repeated for 10 files per leaf and the length of the cell division zone (Ldiv) was estimated by the longest distance between the leaf base and the most distal mitosis, similar to the method of Barlow et al. (1991).

**Kinematic analysis**

The data obtained from the smoothed epidermal cell length profile and the estimated leaf basal meristem length were used in a kinematic analysis, as described by Fiorani et al. (2000).

### Spatial parameters

Local cell density, \( \rho(x) \), at a distance \( x \) from the leaf base, was defined as the reciprocal of local cell length at position \( l(x) \):

\[
\rho(x) = \frac{1}{l(x)} \quad (1)
\]

where position \( x = n \times \Delta x \) with \( n = 1, 2, 3, \ldots \) and \( \Delta x \) is a step of 250 \( \mu \)m, derived from the smoothing procedure described above.

Local cell density was used to calculate the number of cells per file in the growth zone (\( N_{gz} \)) and in the division zone (\( N_{div} \)) as:

\[
N_{gz, div} = \Delta x \times \sum_{n} \left( \frac{\rho(n+1) \Delta x}{2} \right) \quad (2)
\]

where the summation was stopped at the distal margin of the growth zone and of the division zone, respectively (Beemster and Baskin 1998).

The length (\( L_{gz} \)) and number of cells (\( N_{gz} \)) of the elongation-only zone were determined as:

\[
L_{gz} = L_{gz} - L_{div} \quad (3)
\]

\[
N_{gz} = N_{gz} - N_{div} \quad (4)
\]

where \( L_{gz} \) and \( L_{div} \) were obtained as described above.

### Cell division parameters

Owing to the continuous production of new cells in the leaf basal meristem, dividing cells are moved distally through this zone, until they enter the elongation-only zone, where cells stop dividing. During steady-state leaf elongation, the flux of cells through any point in the elongation-only zone is constant and represents the rate of cell production (\( P \), cells d\(^{-1}\)), which was estimated as:

\[
P = \frac{LER}{l_m} \quad (5)
\]

where \( LER \) is leaf elongation rate and \( l_m \) is mature cell length in \( \mu \)m (Silk et al. 1989).

The average cell division rate for the whole leaf basal meristem (\( D \), h\(^{-1}\)) was calculated from the rate of cell production and the number of dividing cells (Beemster and Baskin 1998):

\[
D = \frac{P}{N_{div}} \quad (6)
\]

Considering the exponential nature of the cell division process, the average cell cycle duration (\( T_c \), h) was calculated as (Green 1976; Dubrovsky et al. 1998):

\[
T_c = \ln(2) \times \frac{N_{div}}{P}. \quad (7)
\]

### Temporal parameters

The true residence time of cells in the leaf basal meristem equals the cell-cycle duration, as every dividing cell only exists from the time it was formed until the time the cell undergoes cytokinesis to form two daughter cells. However, if constant cell-cycle duration over time is assumed, the residence time of the most basal cell wall in the division zone (\( T_{div} \), h) can be estimated as (Beemster and Baskin 1998):

\[
T_{div} = T_c \times \log_2(N_{div}) \quad (8)
\]

The residence time of cells in the elongation zone (\( T_{e} \), h) was determined by the number of cells in the elongation zone (\( N_{e} \)) and the flux of cells through that zone which is equivalent to the cell production rate (\( P \)) (Beemster and Baskin 1998):

\[
T_{e} = \frac{N_{e}}{P}. \quad (9)
\]

The duration of cell elongation (\( t(x) \), i.e. the time taken for a cell to be displaced from the start of the elongation-only zone to a location \( x \) was calculated as (Silk et al. 1989):

\[
t(x) = c n(x) \quad (10)
\]

where \( c \) is the cellochron, i.e. the time taken for a cell to be displaced by one position in the elongation-only zone and equals the inverse of the cell production rate \( P \), and \( n(x) \) is the total number of cells between location \( x \) and the start of the elongation-only zone.

### Strain rate

Once cells exit the division zone and enter the elongation-only zone, they stop dividing and continue to elongate until they reach their mature size. The relative cell elongation rate in the elongation-only zone (strain rate, \( r, h^{-1} \)), was calculated from the derivative of the cell-length profile (\( \partial l / \partial x \)) and the cell production rate \( P \) (Silk et al. 1989):

\[
r(x) = P \times \frac{\partial l}{\partial x}. \quad (11)
\]

The corresponding strain-rate profile described the relative cell elongation rate as a function of distance from the leaf base.

### Measurement of cell wall extensibility

A fourth set of 12 plants per species was used to determine the capacity of cell walls to extend upon acidification. The third leaf on the main stem of each plant was harvested within the first 2 d after emergence from the sheath of main stem leaf number two, when the leaves were in the linear growth phase. Leaf segments of 10 mm were excised at a distance from the leaf base where strain rate was around its maximum, and the midvein was removed. The segments were frozen to remove turgor pressure, and thawed again for cell-wall extensibility measurements with a constant stress apparatus (Rayle and Cleland 1972). The leaf strips were placed between two clamps 3 mm apart and submerged in a 50 mM Na-acetate buffer of pH 6.8. Subsequently, a constant tension of 10 g was applied to the tissue, and tissue extension over time was measured with a position transducer. After a constant rate of extension was achieved, the buffer was replaced with a 50 mM Na-acetate buffer of pH 4.5 and the change in extension rate was recorded. The data were expressed as relative extension rate (rate of increase in tissue length over time, per unit of tissue length already present; mm mm\(^{-1} \) h\(^{-1} \)). Owing to occasional breaking of the leaf tissue in *Ae. tauschii*, the sample size was reduced to eight plants in this species.
Statistics

Data were analysed with SPSS 8.0 for Windows statistical software (SPSS, Inc., Chicago, IL, USA). A one-way analysis of variance (at \( \alpha = 0.05 \)) was used to test for significant differences in measured and calculated parameters between \( \textit{Ae. caudata} \) and \( \textit{Ae. tauschii} \).

Results

Leaf elongation

In both species, the increase in length of leaf number three on the main stem was approximately linear during the first 4 d after appearance from the encircling leaf sheath (Fig. 1). For each species, the change in daily LER over this period was on average less than 20% (Fig. 1, inset). We chose to calculate a constant LER per leaf over this steady growth period.

The more than 2-fold difference between \( \textit{Ae. caudata} \) and \( \textit{Ae. tauschii} \) in final length of leaf number three on the main stem was correlated with a similar difference between these species in leaf elongation rate (Table 1 and Fig. 1). The duration of leaf elongation of leaf number three on the main stem was the same in both species (Table 1).

Epidermal cell length distribution

Figure 2 shows the cell-length distribution along the abaxial leaf axis of leaf number three on the main stem in \( \textit{Ae. caudata} \) and \( \textit{Ae. tauschii} \), determined within 2 d after the leaves emerged from the encircling leaf sheath. Cell size in the division zone was the same in both species (approx. 20 \( \mu \)m), and in both species, cells reached the same mature cell size (approx. 250 \( \mu \)m) (Table 1). For the \( \textit{Aegilops} \) species in this study, LER was not associated with mature cell size.

Spatial and temporal dimensions of the growth zone

The total growth zone of \( \textit{Ae. tauschii} \) (22.3 mm) was twice as long as that of \( \textit{Ae. caudata} \) (11.5 mm) (Figs 2, 3A). Also the lengths of the division zone and the elongation-only zone were twice as high in \( \textit{Ae. tauschii} \) (\( L_{\text{div}} = 3.6 \text{ mm} \); \( L_{\text{ez}} = 18.6 \text{ mm} \)) as in \( \textit{Ae. caudata} \) (\( L_{\text{div}} = 1.6 \text{ mm} \); \( L_{\text{ez}} = 9.9 \text{ mm} \)) (Fig. 3A, \( P<0.01 \)). Similar differences between the species were found when the number of cells in the different zones were compared; \( \textit{Ae. tauschii} \) had twice as many cells in the division and elongation-only zone as \( \textit{Ae. caudata} \) (Fig. 3B, \( P<0.01 \)). Although the length of the
Epidermal leaf cell dynamics in two *Aegilops* species

Functional Plant Biology 429

Division zone was smaller than that of the elongation-only zone, cells spent more time in the division zone \( T_{\text{div}} \) than in the elongation-only zone \( T_{\text{ez}} \) (Fig. 3). \( T_{\text{div}} \) was the same in *Ae. caudata* as in *Ae. tauschii* \((P=0.98)\). \( T_{\text{ez}} \) tended to be higher in *Ae. caudata* than in *Ae. tauschii* \((P<0.01)\) but the difference did not result in a difference in residence time in the total growth zone between the species \((P=0.37)\).

**Cell production rate, cell division rate and strain rate**

During steady-state leaf growth, the flux of cells through any point in the elongation-only zone is constant and equals the cell production rate. The cell production rate within cell files adjacent to the stomatal cell files was 2.6 times higher in *Ae. tauschii* than in *Ae. caudata* (Table 1). Differences in cell production rate are determined by differences in the cell division rate and/or the number of dividing cells. The dividing leaf cells of *Ae. caudata* and *Ae. tauschii* had similar cell division rates (Table 1), so they only differed in the number of dividing cells (Fig. 3B).

The derivative of the cell-length distribution along the growth zone (Fig. 2) gives the strain-rate distribution, i.e. the distribution of the relative rates of cell expansion, along the growth zone (Fig. 4A). In both species, the strain rate increased sharply with distance from the leaf base, reached a maximum in the middle of the elongation-only zone, and decreased again to zero at the end of the elongation-only zone. The maximum strain rate was the same for both

**Fig. 3.** Average \((A)\) length of, \((B)\) number of cells in, and \((C)\) cell residence time in the division and elongation-only zones of the abaxial epidermis of leaf number three on the main stem of *Ae. caudata* and *Ae. tauschii*. Vertical bars indicate s.e. for the division and elongation-only zones \((n=5)\). Different letters above vertical bars indicate a significant difference between the growth zones of the species.

**Fig. 4.** Spatial \((A)\) and temporal \((B)\) distribution of strain rate in abaxial epidermal cell files adjacent to stomatal cell files of leaf number three on the main stem in *Ae. caudata* and *Ae. tauschii*. Strain rate data points are derived from the cell length profile (Fig. 2 and Eq. 11) of five leaves per species, and each curve is plotted outside the basal meristematic region. Vertical bars indicate s.e.
species (± 10% h⁻¹) and was therefore, not correlated with LER. Figure 4B shows the strain rate plotted against the duration of cell elongation, i.e. the time a cell spent in the elongation-only zone after leaving the leaf basal meristem. After leaving the leaf basal meristem, leaf cells of Ae. caudata reached their maximum strain rate 9 h later than those of Ae. tauschii, and they also exited the elongation-only zone 10 h later. In other words, the leaf cells of Ae. tauschii moved more rapidly through the elongation-only zone than those of Ae. caudata.

Effects of acidification on cell wall extensibility

Figure 5 shows the relative extension rate of isolated cell walls placed under constant stress of 10 g before and after acidification of the apoplast, for Ae. caudata and Ae. tauschii. The relative cell wall extension rate under constant stress was similar for the two species. Acidification significantly increased the relative cell-wall extension rate in both species. However, the relative cell-wall extension rate after acidification was also similar for both species.

Discussion

Leaf elongation

This study shows that the considerable difference in final length of leaf number three on the main stem between Ae. caudata and Ae. tauschii was entirely due to the difference in leaf elongation rate, whereas leaf elongation duration was similar for the two species. Similar observations were made by Tonkinson et al. (1995) and Calderini et al. (1996) for wheat cultivars; dwarf wheat genotypes showed a slower leaf elongation rate than wild-type genotypes, but the same duration of leaf elongation. Fiorani et al. (2000) also found similar results in a comparison of four Poa species, which differed in final leaf length. In the latter study, however, the visible leaf-elongation duration was determined (time between appearance of the leaf and end of the growth period), as opposed to the estimated leaf-elongation duration determined in the present study. Visible leaf elongation, however, is still an underestimation of the actual duration of leaf elongation. It has been shown that grass leaves elongate much more slowly during the period of invisible growth, and hence the duration of leaf elongation is much longer than that estimated from the LER during the visible growing period (Durand et al. 1999).

Leaf elongation of Ae. caudata and Ae. tauschii was approximately linear during the first 4 d after leaf appearance. This steady-state elongation is required for performing a kinematic analysis based on cell length profiles (Silk and Erickson 1979). The Aegilops leaves in the present study were sampled within 2 d of leaf emergence, during the period of steady-state growth. Schnyder et al. (1990) have shown for Lolium perenne that during this steady growth period, leaf elongation is almost entirely due to blade (not sheath) elongation. Indeed, in the leaf samples in the present study, the ligule (boundary between blade and sheath) was either not yet or only just initiated. However, this does not affect cell-length and strain-rate profiles (Kemp 1980; Schnyder et al. 1990), and the whole growth zone can be treated as a continuous zone for the derivation of kinematic parameters.

It is possible that LER was reduced during the colder night period, as was measured for example by Beemster et al. (1996), thereby overestimating LER and affecting the assumption that leaf growth is in steady state. However, we assumed that both species are similarly affected by the night period and that cell-length profiles are conserved under these changes.

Cell production rate determines differences in leaf elongation rate

On a cellular level, faster leaf elongation rate in Ae. tauschii compared with Ae. caudata was associated with a longer growth zone in which more cells are elongating simultaneously. The elongating cells of Ae. tauschii had a similar maximum elongation rate and a slightly shorter residence time in the elongation zone than Ae. caudata, which resulted in similar mature cell sizes in these species. After reaching a maximum, the strain rate of Ae. caudata decreased sharply with distance from the leaf base to approximately half of the maximum within a 2.5 mm region of the growth zone. In Ae. tauschii however, near-maximum strain rates were maintained within a 5 mm region. When plotted against duration of cell elongation, maximum strain rate was reached earlier in Ae. tauschii than in Ae. caudata, but decreased at similar speeds after that. This means that the leaf cells of Ae. tauschii move through the elongation zone faster and that its wider spread of maximum strain rate is caused by more cells elongating at this maximum rate, and not by cells elongating for a longer period.
The longer growth zone of *Ae. tauschii* was correlated with a higher rate of cell production in the leaf basal meristem, which resulted from a larger number of dividing cells and not from a faster cell division rate. These results agree with those of Fiorani *et al.* (2000), who compared the epidermal cell-length distribution of leaf number seven on the main stem in four *Poa* species. These similar results in *Poa* and *Aegilops* species, both genera of the Poaceae, suggest that differences in leaf growth within the Poaceae may be determined by differences in activity of the leaf basal meristem.

There are two points of view on what determines organ growth rate. According to the spatial or organismal viewpoint, organ growth is determined by the integral of relative cell elongation rate over the length of the growth zone of the elongating organ. From this point of view, cell division is a process that accompanies cell elongation in the meristem and has no other role than subdividing cell volume (Silk and Erickson 1979). We agree that cell division by itself cannot result in leaf elongation. However, we suggest that the higher cell production rate (due to an increase in the number of dividing cells) in the basal meristem of *Ae. tauschii* leaves increased the number of cells elongating at the same time, which was reflected in a longer elongation zone with more expansion potential. From this material or cellular point of view, cells behave as individual material particles that have their own developmental program (Silk and Erickson 1979). However, the cellular control of leaf growth does not exclude the possibility of an organismal control. Genetic evidence for a link between cellular and organismal theories has recently been examined by Tsukaya (2002) in leaf morphology mutants of *Arabidopsis*. These mutants showed that although the size and number of leaf cells affect leaf size, leaf size is, to some extent, uncoupled from the size and number of cells by a compensatory system.

The results of the present study provide indirect evidence for a regulatory role of cell production in leaf elongation rate. More direct evidence showing that organ growth rate is limited by cell production has been provided by Doerner *et al.* (1996), Cockerall *et al.* (2000) and De Veylder *et al.* (2001a; b). In these studies, overexpression of regulatory cell-cycle genes in *Arabidopsis* led to altered organ growth rates. Overexpression of *CKS1At* reduced root growth rates due to an increase in cell-cycle duration and a reduction in root meristem size (De Veylder *et al.* 2001b).

It is interesting to note here that a larger number of parallel cell files was observed in the leaf growth zone of *Ae. tauschii*, the species with the longer leaf basal meristem, whereas the width of the cell files was similar in both species (unpublished). This indicates not only a difference in the number of proliferative divisions (determining number of cells per file) between the species, but also in the number of formative divisions (determining number of cell files). These differences in the number of formative divisions may reflect differences in size of the shoot apex diameter at leaf initiation between the species (Pieters and Van den Noort 1988). Alternatively, they may have resulted from differences in cellular processes taking place during the primordial stages that follow leaf initiation (Beemster and Masle 1996).

### Epidermal cell elongation rate

The strain-rate distribution shows that *Ae. caudata* and *Ae. tauschii* did not differ in their maximum rates of cell expansion. The fact that both species showed the same capacity to extend their cell walls upon acidification supports this observation. Although we compared the extensibility of part of the leaf tissue where cell expansion rate was at its maximum and cell sizes were similar, it is important to note here that we could not exclude a difference in epidermal cell age, and hence cell wall structure, between the species.

Numerous studies on cereal plants have shown that treatment- or species-related differences in leaf growth are positively correlated with differences in cell-wall extensibility (Van Volkenburgh and Boyer 1985; Keyes *et al.* 1990; Matsukura *et al.* 1998; Lu and Neumann 1999). Van Volkenburgh *et al.* (1985b) presented evidence of the involvement of an acid-growth mechanism in the control of cell-wall extensibility in maize. In these studies, however, differences in leaf growth were associated with differences in mature cell length, whereas the *Aegilops* species in this study did not differ in mature cell length. Although the capacity of cell walls to extend upon acidification did not contribute to differences in cell and leaf elongation rate between *Ae. caudata* and *Ae. tauschii*, acidification of the cell wall may still be an important requirement for cell and leaf expansion in these species, through its effect on the activity of cell wall proteins (Okamoto-Nakazato *et al.* 2000; Cosgrove 2001).

The results of the present study show that species-specific differences in leaf growth potential, and maybe also plant growth potential, are not invariably related to differences in cell expansion but can be related to differences in cell production rate only. Therefore, further investigation of inherent differences in leaf growth of closely related species should focus on the mechanisms controlling cell division.

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