Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature

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Abstract
The spatial distributions of leaf expansion rate, cell division rate and cell size was examined under contrasting soil water conditions, evaporative demands and temperatures in a series of experiments carried out in either constant or naturally fluctuating conditions. They were examined in the epidermis and all leaf tissues. (1) Meristem temperature affected relative elongation rate by a constant ratio at all positions in the leaf. If expressed per unit thermal time, the distribution of relative expansion rate was independent of temperature and was similar in all experiments with low evaporative demand and no water deficit. This provides a reference distribution, characteristic of the studied genotype, to which any distribution in stressed plants can be compared. (2) Evaporative demand and soil water deficit affected independently the distribution of relative elongation rate and had near-additive effects. For a given stress, a nearly constant difference was observed, at all positions of the leaf, between the relative elongation rates of stressed plants and those of control plants. This caused a reduction in the length of the zone with tissue elongation. (3) Methods for calculating cell division rate in the epidermis and in all leaf tissues are proposed and discussed. In control plants, the zone with cell division was 30 mm and 60 mm long in the epidermis and in whole tissues, respectively. Both this length and relative division rate were reduced by soil water deficit. The size of epidermal and of mesophyll cells was nearly unaffected in the leaf zone with both cell division and tissue expansion, suggesting that water deficit affects tissue expansion rate and cell division rate to the same extent. Conversely, cell size of epidermis and mesophyll were reduced by water deficit in mature parts of the leaf.

Key words: Maize, leaf expansion rate, cell division rate, cell size, soil water status, evaporative demand, temperature.

Introduction
In monocotyledon leaves, cell division and tissue expansion are limited to the first centimetres beyond the leaf insertion point (Schneider and Nelson, 1988; Durand et al., 1995). The spatial distribution of tissue expansion rate is affected by soil water deficit, with the involvement of root-sourced ABA (Saab et al., 1992; Bacon et al., 1998), and by evaporative demand which probably involves a hydraulic signal (Ben Haj Salah and Tardieu, 1997; Munns et al., 2000). A combination of spatial analyses of expansion rate and of the activities of candidate enzymes can be a powerful tool to identify biochemical mechanisms involved in the control of growth (McQueen Mason et al., 1992; Pritchard et al., 1993; Palmer and Davies, 1996). The same approach could be used for cell division (Schuppler et al., 1998), but a quantitative analysis of the effect of water deficit on the spatial distribution of cell division rate has not been reported yet for monocot leaves, unlike the case of roots (Sacks et al., 1998) or of dicot leaves (Granier and Tardieu, 1999).

Spatial analysis of gene expression in growing tissues combined with spatial analyses of expansion rate and cell division rate can identify genes involved in these controls (Saab et al., 1995; Schünmann et al., 1997). This will

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probably become more and more feasible as the use DNA arrays develops, allowing analysis of the expression of a large number of genes (Spellman et al., 1998). Correspondence between gene expression and tissue expansion rate or cell division rate could therefore be analysed by factorial analysis, in tissues differing by age and degree of stress. The limiting factor for developing such an approach may increasingly be the methodological difficulties in the spatial analyses of cell division rate and of tissue expansion rate.

The determination of relative elongation rate is straightforward. However, the groups who carry out a spatial analysis of enzyme activities or of gene expression may not be expert in kinematic analysis. It is essential that they can compare observed spatial distributions with a robust reference distribution corresponding to the environmental conditions of the experiment. An attempt is made here to model the spatial distribution of relative elongation rate of maize and their joint response to temperature, soil water deficit and evaporative demand, in order to unify tendencies observed over several experiments carried out in contrasting conditions.

The problem is more serious for the determination of cell division rate using the continuity equation (Gandar, 1980; Silk, 1992). This method can cause appreciable errors when environmental conditions and leaf elongation rate are changing with time, which is usually the case in experiments with water deficit. In addition, organs develop in three dimensions, generating difficulties in the use of the continuity equation (Maurice et al., 1997; Liang et al., 1997). Methods to solve these problems are proposed here, and the resulting distributions of cell division rate in well-watered and stressed plants, in the epidermis and in all leaf tissues, are presented.

Theoretical considerations

Working in fluctuating temperature: the use of thermal time

A way to model the effects of non-stressing temperatures (i.e. those which cause neither chilling nor heat shock) is the use of thermal time (Chapman et al., 1993). This concept can be used in the case of leaf development when leaf elongation rate is linearly related to leaf temperature. In this case,

\[
dL/dt = a(T - T_0)
\]

where \( L \) is leaf length, \( T \) is current leaf temperature, \( a \) and \( T_0 \) are the slope and the x-intercept of the relationship between \( dL/dt \) and \( T \). In maize, the relationship between leaf elongation rate and leaf temperature is linear and common to a series of experiments (Ben Haj Salah and Tardieu, 1995, 1996). In particular, the x-intercept of the relationships \( (T_0) \) was 10 °C in all experiments. Equation 1 is therefore valid in a large range of environments and can be integrated. It follows that the increase in leaf length (\( \Delta L \)) during a time interval \( \Delta t \) is:

\[
\Delta L = \int_0^\Delta t (T - T_0)\, dt
\]

where \( \int_0^\Delta t (T - T_0)\, dt \) is thermal time (unit: °C when calculated with a daily timestep). This formalism implies that leaf elongation during the interval \( \Delta t \) only depends on the cumulated temperature above 10 °C, and not on the way in which this cumulated temperature was obtained. For instance, the change with time in leaf length will be the same after 1 degree-day whether the plant has spent 24 h at 11 °C (1 °C warmer than 10 °C), 12 h at 12 °C, 3 h at 18 °C or 1 h at 34 °C. This is generally the case in maize, as it is in sunflower and sorghum but with different \( T_0 \) (Granier et al., 1998; Lafarge et al., 1998).

Thermal time is, therefore, a representation of time as it is sensed by the plant. It is shown in the ‘Results’ section that expressing elongation rate per unit thermal time allows unifying results of a series of experiments, so resulting data are unique for a given genotype regardless of temperature.

Can the continuity equation be used for calculating cell division rate when leaf elongation rate changes with time?

In monocotyledons, cells are produced continually near the leaf insertion point and pushed forward by younger cells. The deposition rate of any element can be deduced from the spatial distributions of local elongation rate and of the concentration of this element (Gandar, 1980; Silk, 1992). This method can apply to cells, thereby allowing the estimation of the rate of cell deposition per unit time in any zone of the growing leaf. It is based on the calculation of the cell flux that crosses a small element of the leaf (e.g. the number of cells per hour which crosses a 1 mm wide leaf strip located at distance \( i \) from the leaf insertion point and perpendicular to the midrib). The change with time in cell number in this element \( (dp/dt) \), cell mm\(^{-1}\)h\(^{-1}\), equals the difference in cell fluxes which enter and which quit this element \( (dJ/dx)_i \), plus the number of new cells which appear in the element (cell deposition rate, \( d_i \)).

\[
(dp/dt)_i = -(dJ/dx)_i + d_i
\]

where \( J \) is the cell flux and \( p \) the cell density. The cell flux \( (J) \) is given by the product of cell density \( (\rho_i) \) by the local rate of cell displacement \( (v_i) \) so:

\[
d_i = (dp/dt)_i + (d(Jv)/dx)_i
\]

\[
d_i = (dp/dt)_i + \rho_i(dv/dx)_i + v_i(dp/dx)_i
\]

Equation 5 is the classical form of the continuity equation. The term \( (dv/dx)_i \) is the relative elongation rate of element
i (RER). The relative division rate (RDRi, cells per cell and h) is:

$$\text{RDR}_i = \frac{d_i}{\rho_i}$$

(6)
time interval or on characteristics of cell flux because it is a simple balance of cell number. In contrast, the use of Equations 4 and 5 should be limited to very short times or to the case when cell flux is constant. A simple simulation can show that the mean flux (di) during the period considered appreciably differs from the product of mean density (ρi) by mean velocity (vi) when this is not the case. In practice, 4–6 h are necessary to observe a measurable displacement in the leaf. It is essential that the displacement rate does not change with time during this period (steady-state). This was the case in the experiments reported here.

In addition, it is suggested that leaf elongation rate should be stable with time for a longer period (typically 1 or 2 d) to avoid appreciable errors. If leaf elongation rate changes with time, changes in displacement rate can be detected with precision (see Materials and methods), but changes in cell density are more difficult to detect given the large variability of cell density (see Fig. 6). They will not be detected before at least 1 or 2 d, thereby causing large errors. It is relatively easy to get a steady-state in maize leaves during their linear period of elongation if plants are well watered and subjected to a stable environment (e.g. Fig. 2 in Ben Haj Salah and Tardieu, 1995). This is not the case in plants subjected to naturally fluctuating temperature, evaporative demand or soil water deficit. It is shown in the ‘Results’ section that Equation 5 can be used in fluctuating temperature when rates are expressed per unit thermal time, but only when evaporative demand and soil water status are maintained constant during the experiment.

Materials and methods

Plant culture and measurements of growth conditions

Maize (Zea mays L., F1 cv. DEA) plants were grown in a greenhouse in Montpellier (southern France) for six experiments. Seeds were sown in columns (0.1 m diameter, 0.5 m height) containing a 1:1 mixture (v/v) of a loamy soil and an organic compost. They were irrigated with modified one-tenth strength Hoagland solution supplemented with minor nutrients. Additional light in the greenhouse was provided by a bank of sodium lamps maintaining a photoperiod of at least 14 h. Light was measured continuously using a photosynthetic photon flux density (PPFD) sensor (LI-190SB, LI COR, Lincoln, Nebraska, USA). Air temperature and relative humidity were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland). Leaf temperature was measured with a copper–constantan thermocouple (0.4 mm diameter) inserted in the meristem. All measurements of temperature, PPFD and relative humidity were averaged and stored every 600 s in a datalogger (Campbell Scientific, Ltd, CR10 Wiring Panel, Shepshed, Leicestershire, England). Predawn leaf water potential was measured before dawn. Six mature leaves per treatment were excised and placed in a pressure chamber for measurement.

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Water deficit (WD1, WD2 and WD3), evaporative demand (VPD) and temperature (Temp1 and Temp2) experiments

Experiment WD1: Plants were grown in July 1994 in 50 columns. Mean air temperature in the greenhouse was 24°C and 32°C during night and day, respectively, and daily PPFD was 25 mol m⁻² d⁻¹. When leaf 5 emerged, watering was stopped on half of the columns so soil water potential continuously decreased, while the other half continued to be fully irrigated (predawn leaf water potential of −0.1 MPa). Leaf elongation rate decreased in water-deficient plants, but completely recovered 1 h after rewatering (Ben Haj Salah and Tardieu, 1997).

Experiments WD2 and WD3: Plants were grown in November 1998 (WD2) and February 1999 (WD3) in 80 columns. Mean air temperature during Experiments WD2 and WD3 were, respectively, of 19.5°C and 22°C. Columns were weighted once a day, before each watering. This allowed calculation of the volume of nutrient solution required to maintain soil water content at a constant value. In the watered treatment, soil water content was maintained at 0.6 g g⁻¹ dry soil until the emergence of leaf 6 and at 0.75 g g⁻¹ later on. In the water deficit treatment, watering was stopped at plant emergence until soil water content reached 0.31 g g⁻¹, and was managed afterwards in order to maintain it constant until the end of the experiment. In well-irrigated plants, leaf predawn water potential ranged from −0.16 to −0.10 MPa. It was −0.31±0.17 and −0.25±0.10 in water-deficient plants of Experiments WD2 and WD3, respectively.

Experiment VPD: Plants were grown in April 1999 in 50 columns which were maintained at soil water retention capacity. When the 6th leaf emerged, plants were transferred to a growth chamber. Environmental conditions in the growth chamber were measured with the same methods and time-lapse as in the greenhouse. Air temperature in the growth chamber was automatically regulated in such a way that leaf temperature was kept at a constant value of 25°C. Light in the growth chamber was provided by a bank sodium lamps with a constant PPFD of 1000 µmol m⁻² s⁻¹ for a photoperiod of 16 h (daily PPFD of 57.6 mol m⁻² d⁻¹). Air VPD was maintained at 2 kPa during the studied 48 h period.

Experiments Temp1 and Temp2: Plants were sown in the greenhouse in August 1994 (Experiment Temp1, Ben Haj Salah and Tardieu, 1995) and December 1998 (Experiment Temp2). Columns were transferred to a growth chamber after the emergence of the 6th leaf. Each batch of plants was then grown at constant temperature during a 48 h period (21, 26, 30, and 35°C for August 1994 and 19°C and 26°C for December 1998). Light in the growth chamber was provided by a bank of cool-white fluorescent tubes with a constant PPFD of 230 µmol m⁻² s⁻¹ with continuous light in Experiment Temp1 and for a photoperiod of 16 h in Experiment Temp2 (daily PPFDs of 19.9 and 13.3 mol m⁻² d⁻¹). Air VPD was maintained at values below 1 kPa.

Spatial distribution of relative elongation rate (RER): The displacement of needle holes in the basal part of the leaf was followed using a method presented earlier (Ben Haj Salah and Tardieu, 1995) in five plants per treatment. The duration of displacement studied was 6 h in Experiment Temp1, and 4 degree days in all other experiments, corresponding to 10, 6 and 4 h at 19°C, 26°C and 35°C, respectively. A period of 6 h corresponded to very short displacements of points at 14°C and 17°C in Experiment Temp1, so calculated relative elonga-
tion rates were inaccurate. Values reported in Ben Haj Salah and Tardieu (Ben Haj Salah and Tardieu, 1995) at these two temperatures were therefore not considered here. Briefly leaf elongation zone was marked with 40 needle holes (0.2 mm diameter). The elongation of leaf strips between two neighbouring holes was obtained by subtracting the initial from the final distances between the holes. The initial distance was estimated by measuring (image analysis, Bioscan-Optimas V 4.10, Edmonds, USA) the distance between corresponding neighbouring holes on the sheath of the 3rd (non-growing) leaf. Sixth leaves were then freed from enclosing leaves, and final positions of needle marks were recorded with the image analyser. Relative elongation rate of the ith leaf strip \( \text{LER}_{i} \) (mm mm\(^{-1}\) h\(^{-1}\)) was calculated as:
\[
\text{LER}_{i} = \frac{(\Delta L_{i,0} - \Delta L_{i,1}) + \text{LER}_{i-1}}{(\Delta L_{i,0} - \Delta L_{i,1}) + \text{LER}_{i-1} + \text{LER}_{i+1}}
\]
where \( \Delta L_{i,0} \) and \( \Delta L_{i,1} \) (mm) are the initial and final distances between holes \( i \) and \( i+1 \). \( \text{LER}_{np} \) (mm h\(^{-1}\)) is the mean elongation rate of non-pierced plants and \( \text{LER}_{cum} \) (mm h\(^{-1}\)) is the sum of elongation of all leaf strips of pierced plants, divided by the duration of the experiment (\( \Delta t \)). The ratio \( \frac{\text{LER}_{np}}{\text{LER}_{cum}} \) corrects \( \text{LER} \) for the effect of piercing injury.

**Spatial distribution of relative cell division rate in the epidermis**

Cell division rate was calculated according to Equations 5 and 6. Local displacement rates were deduced from the spatial distribution of relative elongation rate. Epidermal cell density \( \rho \) in a leaf strip was evaluated in cell files and estimated as the reciprocal of the mean epidermal cell length in the strip. The spatial distribution of cell length was measured before and after the measurements of relative elongation rate in some experiments. It was found to be stable during this short period (\( \frac{d\rho}{dt} = 0 \) in Equations 4 and 5), so it was only measured after measurement of relative elongation rate in most experiments. A 4% (w/w) solution of polyvinyl formaldehyde in chloroform (Experiments Temp1 and WD1) or a nail varnish (other experiments) was spread on the upper epidermis in the basal 200 mm of the sixth leaf, after the leaf was freed from enclosing leaves. A transparent negative film of upper epidermis cells was obtained after evaporation of the chloroform or of the varnish. Films were placed under a microscope (LEICA-Leitz DM RB, Wetzlar, Germany) coupled to the image analyser. The lengths of epidermal cells were measured at the position of each needle hole (about every 5 mm) from 1–20 mm from the leaf insertion point, and every second needle hole (about every 10 mm) from 20–100 mm. Three sets of 30 cells were measured at each position of three leaves per treatment. Mean length, standard deviation, maximum and minimum cell lengths were recorded in each cell set. Files taken into account were close to the midrib and had a negligible angle with it.

**Spatial distribution of relative cell division rate in all leaf tissues (epidermis + mesophyll):** Equation 5 was still used, but the term \( \rho \) stood here for the cell number in a leaf strip located at a given distance from the leaf insertion point (\( \rho \)). Five 6th leaves per treatment were harvested and cut in eight 1 cm strips, from the leaf insertion point. Cell number in each leaf strip was determined after digestion in a solution of chromic acid 20% 12 h at 21 °C on a shaker and counting the number of cells under a microscope (LEICA-Leitz DM RB, Wetzlar, Germany) with a haemocytometer (modified from Milthorpe and Newton, 1963).

In this case, the term \( \frac{d\rho}{dt} \), is not null because of the increase with time in width of the considered leaf strip. However, the precision of the method for determining \( \rho \), combined with the plant to plant variability, did not allow a proper estimation of \( \frac{d\rho}{dt} \). The latter was therefore estimated by considering that the cell number per unit leaf area \( \rho_{i} \) did not change during the 6 h period considered for estimation of relative elongation rate and that the thickness of the leaf blade did not change during the same period. Both assumptions were based on preliminary measurements (not shown). \( \rho_{i} \) is the product of \( \rho \) by the width of the considered leaf strip \( W \), so,
\[
\frac{d\rho_{i}}{dt}_{i} = \rho_{i} \frac{dW}{dt} + \frac{W}{dt} \rho_{i} \text{RWR}
\]
The second term of Equation 8 is null because it is assumed that \( \rho_{i} \) does not change during the measurement period, so.
\[
\frac{d\rho_{i}}{dt}_{i} = \rho_{i} \frac{dW}{dt} + \frac{W}{dt} \rho_{i} \text{RWR}
\]

**Results and discussion**

**Modelling the spatial distribution of relative expansion rate in leaves of plants experiencing fluctuating temperature, soil water status and evaporative demand**

**Alternation of relative elongation rate during day and night in fluctuating conditions:** Spatial distributions of \( \text{RER} \) was analysed during day and night periods in Experiment WD1 (Fig. 1). It was affected by soil water status during both day (Fig. 1a) and night (Fig. 1b), but with a greater effect during the day. In plants at a predawn leaf water potential of \(-0.4\) MPa, leaf zones located beyond 30 mm from the insertion point ceased to grow during the day, but were still growing during the night. The same alternation was observed beyond 50 mm in plants at a predawn leaf water potential of \(-0.25\) MPa. However, it is not possible to compare distributions of relative elongation rates observed during day and night periods because plants were at different temperatures.

The distribution of relative elongation rate is unique regardless of temperature if expressed per unit thermal time, in the absence of soil water deficit and evaporative demand: At each position in the leaf, local relative elongation rates increased linearly with meristem temperature in the series of treatments of Experiments Temp1 and Temp2 (Fig. 2a). For example, maximum relative elongation rate (observed at 20–35 mm from the leaf insertion point) was linearly related to meristem temperature with a relationship which applied to Experiments Temp1 and Temp2 (growth chamber) and also to experiments in the greenhouse and in the
Plants with moderate water deficit at the 0.05 probability level (and (b) was reduced in plants subjected to water deficit. Experiments Temp1 and Temp2. Interval of confidence measured in the leaf strip between 20 mm and 35 mm. Symbols ence distribution of temperature. Inset (c) Relationship between meristem temperature and obtained by subtracting a constant value from the refer-
\[
RER_{i,j} = a(T_j - T_0)
\]
where \( RER_{i,j} \) is the relative elongation rate observed in position \( i \) and time \( j \), \( a \) is the slope of the linear relationship between \( RER \) and temperature in point \( i \), \( T_j \) is current meristem temperature at time \( j \) and \( T_0 \) is the x-intercept of the linear relationship between \( RER \) and \( T \) (Fig. 2c). \( T_0 \) was equal to 10°C, consistent with earlier results (Ben Haj Salah and Tardieu, 1995). Each distribution could therefore be deduced from any other by multiplying every local value by a temperature-dependent factor (affine transformation).

Following the reasoning presented in the ‘theory’ section, relative elongation rate can be expressed per unit thermal time. Expressed in this way, the distribution of \( RER \) was common to all studied temperatures in different experiments carried out over several years. The local \( RER \) at any position \( i \) in the leaf and any temperature can therefore be obtained as.
\[
RER_{i,j} = RER_{ref,i} \times (T_j - T_0)/24
\]
where \( RER_{i,j} \) (mm mm\(^{-1}\) h\(^{-1}\)) is the local \( RER \) observed in position \( i \) and time \( j \). \( RER_{ref,i} \) (expressed per unit degree day, mm mm\(^{-1}\) C\(^{-1}\)) is the \( RER \) at position \( i \) in the mean distribution deduced from Fig. 2b (called reference distribution hereafter). \( T_j \) and \( T_0 \) have the same meaning as in Equation 11. The reference profile is therefore a stable characteristic of the studied genotype, valid for several experiments provided that plants experience no stress. It is reported in Figs 3, 4 hereafter, which present the results of independent experiments.

A constant soil water deficit decreased relative elongation rate by a constant difference in all positions of the leaf, under low evaporative demand: A constant soil water deficit with low evaporative demand affected local relative elongation rates at all distances from the leaf insertion point in Experiments WD2 and WD3 (Fig. 3a, b). Control plants in both experiments had a distribution of \( RER \) per unit thermal time which closely followed the reference distribution calculated from Fig. 2b (dotted lines, Fig. 3a, b). In both experiments, a nearly constant difference was observed between \( RERs \) in controls and stressed plants at every distance from the leaf insertion point. This can be visualized in Fig. 3a and b by comparing observed data in stressed plants with the solid line obtained by subtracting a constant value from the reference distribution of \( RER \). This difference was slightly larger in Experiment WD1 than in WD2 (−0.045 versus −0.04 mm mm\(^{-1}\) C\(^{-1}\)) consistent with a lower predawn leaf water potential in Experiment WD1. As a consequence, the length of the zone with tissue elongation was reduced in plants subjected to water deficit, and \( RER \)
Experiment VPD, predawn leaf water potential and the concentration of ABA in the xylem sap were close to 0, but leaf elongation rate was still reduced (Ben Haj Salah and Tardieu, 1997). As in the case of water deficit, observed data were close to an overall tendency obtained by subtracting 0.04 mm mm$^{-1}$ h$^{-1}$ from the reference distribution of $RER$ calculated from Fig. 2b (solid line in Fig. 3b). In this case, the distribution more closely followed the tendency than in the case of soil water deficit, without a systematic bias toward higher values of $RER$ at proximal positions of the leaf and lower $RER$s at distal positions. An equation similar to Equation 4 can therefore be written for the effect of evaporative demand.

$$RER_{ij} = RER_{ref,i} - c(VPD)$$

where $c(VPD)$ is the reduction in relative elongation rate due to an increase in evaporative demand. This term was constant over the whole profile.

**Combined effects of decreasing soil water status and fluctuating evaporative demand:** The above results allow the reinterpretation of the night and day distributions of $RER$ presented in Fig. 1, obtained in an experiment where soil water status continuously declined with time under a naturally fluctuating evaporative demand. At the beginning of the experiment (Fig. 4a), when predawn leaf water potential was close to $-0.1$ MPa, relative elongation rates measured during the night were close to the reference distribution calculated from Fig. 2b. Day-time $RER$s expressed per unit degree day were lower than this reference distribution, indicating that the daily increase in evaporative demand in the greenhouse had a depressing effect. This effect was appreciable in the 10–80 mm zone, but not in the first 10 mm. When predawn leaf water potential reached $-0.25$ MPa, both day and night $RER$s were affected, but day $RER$s were always lower than night $RER$s. The same conclusion applied to a later period of the experiment, when predawn leaf water potential reached $-0.4$ MPa. Consistent with Equations 4 and 5, distal zones of the leaf stopped growing at a distance from leaf insertion point which decreased with increasing water deficit or evaporative demand. However, the tendency towards a lesser effect near the meristem than at further distances was more marked in this experiment than in those presented in Fig. 3.

**Comparison of the effects of temperature, soil water deficit and evaporative demand:** A clear difference was observed between the effect of temperature on one hand (Fig. 2) and those of soil water deficit or evaporative demand on the other hand (Figs 3, 4). Changes in temperature (Fig. 2) affected relative elongation rates at all positions of the leaf by a constant ratio, so all profiles corresponded to an affine transformation of a unique reference profile (Equation 3). As a consequence, the length of the zone with tissue elongation was not affected by temperature.

**High evaporative demand decreased relative elongation rate by a constant difference in all positions of the leaf, in the absence of soil water deficit:** A high and constant evaporative demand without soil water deficit caused approximately the same effect on the spatial distribution of $RER$ than a change in soil water status (Experiment VPD, Fig. 3b). It has been shown that, in conditions similar to those in

![Fig. 3. Effect of soil water deficit (a, b) and of evaporative demand (b) on the spatial distribution of relative elongation rate (RER) in the elongating zone of 6th maize leaf, expressed per unit thermal time. (●) Well-watered plants. (◇) Plants subjected to a moderate water deficit and near-zero VPD in Experiments WD2 (a) and WD3 (b). (○) Well-watered plants subjected to a continuous VPD of 2 kPa (Experiment VPD). Predawn leaf water potential was $-0.31$ and $-0.25$ MPa in Experiments WD2 and WD3. Dotted lines in panels (a) and (b) represent the mean spatial distribution of $RER$ presented in Fig. 2b. Solid lines in panels (a) and (b) are translations of the dotted line measured during the night were close to the reference distribution calculated from Fig. 2b (solid line in Fig. 3b). In this case, the distribution more closely followed the tendency than in the case of soil water deficit, without a systematic bias toward higher values of $RER$ at proximal positions of the leaf and lower $RER$s at distal positions. An equation similar to Equation 4 can therefore be written for the effect of evaporative demand.

$$RER_{ij} = RER_{ref,i} - c(VPD)$$

where $c(VPD)$ is the reduction in relative elongation rate due to an increase in evaporative demand. This term was constant over the whole profile.
In contrast, soil water deficit and increase in evaporative demand (Figs 3, 4) decreased relative elongation rates at all positions of the leaf by a nearly constant difference (translation of profiles towards lower values), except in the zone closest to the leaf insertion point. This caused a reduction in the length of the zone with tissue elongation. These findings are consistent with results of Pritchard et al. (Pritchard et al., 1990a, b) on cereal roots. A change in temperature affects tissue extensibility (slope of the relationship between turgor and elongation rate), while an osmotic stress increases the yield threshold (x-intercept of this relationship). It could therefore be expected that changes in temperature affect relative elongation rate by a constant ratio, while a water stress affects it by a constant difference.

Combined effects of temperature, water deficit and evaporative demand on the spatial distribution of relative elongation rate: It is confirmed here that soil water status and evaporative demand have independent effects on maize leaf expansion rate, independent of the effect of temperature. This is consistent with the result of a temporal analysis carried out on whole-leaf elongation rate, in which these effects were additive (Ben Haj Salah and Tardieu, 1997). This view can be extended here for the spatial distribution of relative elongation rate except in the proximal 10 mm of the leaf (Fig. 4). Equations 3–5 can be combined in order to predict the spatial distribution of relative elongation rate in leaves of maize plants subjected to joint changes in temperature, water deficit and evaporative demand.

\[
RER_{i,j} = (RER_{ref,i} - b(\Psi) - c(VPD)) \times (T - T_0)/24
\]

where \(RER_{i,j}\) (mm mm\(^{-1}\) h\(^{-1}\)) is the \(RER\) observed in position \(i\) on day \(j\), and other variables are defined as in Equations 2–4. \(b(\Psi)\) and \(c(VPD)\) are reductions in relative elongation rate due to soil water status and evaporative demand, respectively, and can be deduced from the results on whole leaf elongation rates (Ben Haj Salah and Tardieu, 1997). The profile of relative elongation rate observed in a given experiment can therefore be calculated in any situation provided that growth is affected by no environmental condition other than temperature, soil water status and evaporative demand. However, this model failed in the first 10 mm near the leaf insertion point in two occasions in Experiment WD1 (Fig. 4a, b). It remains to be clarified whether this is a reproducible tendency, or if it was due to experimental errors in this experiment.

It has been argued earlier (Tardieu et al., 1999) that light has probably no effect per se on the distribution of relative elongation rate when leaf is autotrophic and has large reserves of carbon. Apparently positive effects of light, consistent with results of Milthorpe and Newton (Milthorpe and Newton, 1963) and Dale (Dale, 1965) are accounted for by an increase in leaf temperature (by up to 5°C) when leaves are illuminated. Apparently negative effects of light, similar to results of Volenec and Nelson (Volenec and Nelson, 1982) and Schnyder and Nelson (Schnyder and Nelson, 1988) are accounted for by an increase in leaf-to-air VPD, consistent with the results of Shackel et al. (Shackel et al., 1987) who report an acceleration of expansion rate of Vitis vinifera leaves when transpiration decreases.

Spatial distribution of cell division rate and cell size in leaves of maize plants experiencing soil water deficit

Can cell division rate be calculated under fluctuating temperature or evaporative demand?: Conditions are obviously non-steady if relative elongation rate slows down during the night and accelerates during the day because of changes in temperature. Expressing rates per unit thermal time allows the solving of this problem, because the spatial distribution of relative elongation rate is independent of temperature if expressed in this way (Fig. 2b). The displacement rate of cells is proportional to temperature, so it takes twice as much time for a cell to cross the elongation zone at 20°C than at 30°C (72 versus 36 h).

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![Graph showing spatial distributions of relative elongation rate in maize leaves](image-url)
The effects of meristem temperature on relative elongation rate and on displacement rate therefore compensate, giving unique spatial distributions of both RER and cell density regardless of temperature (Ben Haj Salah and Tardieu, 1995). The growing zone of maize leaves can therefore be considered in steady-state even in fluctuating temperature.

Soil water deficit has a higher effect during the day than during the night if evaporative demand is appreciable, so distal zones in the leaf stop growing during the day and resume growth during the night (Figs 1, 4). As a consequence, a steady-state cannot be achieved if evaporative demand is varying with time. This cannot be solved via any transformation of variables because of the alternation of expansion and arrest of expansion in distal zones (Fig. 1). Therefore, it is proposed that calculation of cell division rate needs constant evaporative demand and soil water status during at least the duration of measurement of relative elongation rate (about 6 h), and for a longer period to avoid the numerical problems reported in the ‘theory’ section. In the experiments reported here, leaf elongation rate was constant for at least 30 °Cd (72, 48 and 36 h at 20, 25 and 30 °C ) prior to analysis, i.e. the time required for a cell to cross the elongating zone.

Distributions of cell length and cell division rate in the leaf epidermis: Data for calculating cell division rate in the epidermis are presented in Fig. 5 for Experiment WD2 (Fig. 3a). Leaf elongation rate expressed per unit thermal time was equal during the day and night in both treatments, so the continuity equation could be used in a rigorous way. The results of Experiment WD1 in which expansion rate was fluctuating between day and night are also presented (Fig. 5d).

In control plants of Experiment WD2, epidermal cell division occurred in the proximal 30 mm beyond the leaf insertion point and was maximum in the first 20 mm (Fig. 5c). The same result was observed in Experiment WD1 in which cell division rate was higher because meristem was warmer (Fig. 5d). Cell length increased with distance to the leaf insertion point, and reached 120 μm in average at 70 mm (Fig. 5b). This distribution was similar in all studied leaves (small standard deviation of mean values corresponding to each leaf), but had a large variability at each distance in each leaf. In the 0–20 mm zone, mean cell length was close to 25 μm, while minimum and maximum lengths were 18 and 50 μm, respectively. Cell length remained stable on average in this zone and oscillated between near-constant minimum and maximum values because relative division rate was close to relative expansion rate. Cell length increased at further distances, where the relative expansion rate exceeded the relative division rate, and reached a maximum at 70 mm where expansion ceased. A 2-fold variability in cell length still existed in this zone and in mature zones of the leaf, although it could not be attributed to different positions of cells in the cell cycle.

In plants under water deficit, cell division rate was lower than in control plants and stopped at a closer distance from the leaf insertion point (Fig. 5c, d). In the 0–20 mm proximal zone, cell division rate was more affected than relative expansion rate, so there was a tendency towards longer cells in the water deficit treatment (Fig. 5b). Minimum cell lengths were similar in both treatments but maximum cell lengths were slightly higher with water deficit, suggesting that mitosis occurred with longer cells in the water deficit treatment. Mean cell length increased beyond 20 mm and reached a lower final value than in control plants, because of the lower relative expansion rate in the 30–60 mm zone of the leaf.

Distributions of cell density and cell division rate in all leaf tissues (epidermis plus mesophyll): Cell division rate in the whole leaf and data for calculating it are presented in Fig. 6, for the same batches of plants presented in Fig. 5. Cell division occurred in the proximal 70 mm
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0–20 mm beyond the leaf insertion point but remained appreciable until 60 mm (Ben Haj Salah and Tardieu, 1995). The activity of the p34cdc2 kinase was appreciable in the same leaf zones, and was not observed beyond 70 mm (Granier et al., 2000). As far as is known, this is the first time that cell division rate is calculated with a kinematic method in all tissues of monocotyledon leaves.

Coordination between cell division rate and tissue expansion rate in control and stressed plants: A striking result is that the distribution of cell size was almost unaffected by water deficit in the zone with cell division, both in the epidermis and in the whole leaf. A slight tendency existed toward longer cells in stressed than in watered plants, consistent with the results of Sacks et al. (Sacks et al., 1998) for maize roots subjected to water deficit. This suggests that cell division rate is closely co-ordinated to tissue expansion rate in maize leaves subjected to water deficit, as it is in sunflower leaves subjected to deficits of carbon or of water (Granier and Tardieu, 1998, 1999), but that cell division rate may be slightly more affected than tissue expansion rate.

Conclusion

The synthesis of several experiments leads to robust patterns.

(a) The spatial distribution of relative elongation rate is independent of temperature if expressed per unit thermal time, because meristem temperature affects all local relative elongation rates by a common ratio which is proportional to temperature. The resulting mean distribution of relative elongation rate can be considered as a reference characteristic of a given genotype, to which any distribution in stressed plants can be compared. It can also be used for comparing genotypes in a series of experiments carried out at different temperatures.

(b) Soil water deficit and an increase in evaporative demand independently affect the distribution of relative elongation rate, and behave as having near-additive effects in naturally fluctuating conditions. They both tend to reduce relative elongation rate by a common difference which applies to all positions in the leaf, resulting in a reduction in length of the zone with tissue elongation. This confirms the results of a temporal analysis of the effects of evaporative demand and soil water deficit on leaf elongation rate (Ben Haj Salah and Tardieu, 1997). The resulting simple additive model (Equation 15) allows the responses of genotypes to soil water deficit and evaporative demand to be compared in the field or the greenhouse. This approach is currently being used by the authors in a QTL analysis.

(c) Cell division occurs in a zone which is twice as long in the mesophyll than in the epidermis, in both well-watered and water-deficient plants. Soil water deficit reduces this length in both tissues by about the same

Fig. 6. Spatial distributions of all-tissues cell density and cell division rate in all tissues of 6th leaves of watered and water-deficient maize plants, and elements for calculation of cell division rate. (a) Spatial distributions of relative elongation rate (RER) per unit clock time. (b) Spatial distribution of the relative increase in width (RWR). (c) Distribution of cell density per mm leaf. (d) Relative cell division rate (RDR). Experiment WD2, symbols as in Fig. 5.

Comparison of cell division rates in the epidermis and in the whole leaf: Cell division occurred over a longer zone (and therefore over a longer duration) in whole leaf tissues than in the epidermis. This result is consistent with indirect evaluations of cell division in maize leaves. The proportion of nuclei in S phase is maximum in the
extent. Expansion and cell division occur in the same zones in the mesophyll, but not in the epidermis. As a consequence, spatial analysis cannot help in classifying genes specifically involved in either cell division or tissue expansion, unless analyses are taken to the tissue level.

(d) Cell division rate and tissue expansion rate are closely co-ordinated because cell length in the epidermis and cell density in the mesophyll did not differ by large extents in the leaf zone where both cell division and cell expansion occur. This suggests that both processes may have a common regulation.

References


Granier C, Inzé D, Tardieu F. 2000. Spatial distribution of cell division rate can be deduced from that of p34<sup>cdc2</sup> kinase activity in maize leaves grown at contrasting temperatures and soil water status. Plant Physiology (in press).


