The decrease in growth of phosphorus-deficient maize leaves is related to a lower cell production

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ABSTRACT

The spatial distribution of leaf elongation and adaxial epidermal cell production in leaf 6 of maize (Zea mays L. cv. Cecilia) plants grown in a growth chamber under two contrasting availabilities of P in the soil was investigated. Lower displacement velocities from 32.5 mm from leaf base and a shorter growth zone were found in low P (LP) leaves compared with control leaves. P deficiency significantly diminished maximum relative elemental growth rate and shifted its location closer to the leaf base. Cells were significantly longer in LP than in control leaves for all positions from the leaf base except at the end of the growth zone. For both treatments it took a similar time for a cell situated at the leaf base to reach the limit of the growth zone. The average length of the cell division zone was decreased by 21% in LP leaves. Significant differences were found in cell production and cell division rates from 12.5 mm from the leaf base although maximum values were similar between P treatments. A shorter zone of cell division with lower cell production rates along most of its length was the regulatory event that decreased cell production, and ultimately leaf elongation rates, in P-deficient maize plants.

Key-words: Zea mays L.; cell division rate; cell flux; division zone; epidermal cell length; growth zone; leaf elongation rate; maize; phosphorus; relative elemental growth rate.

INTRODUCTION

The reduction in plant biomass accumulation and in leaf surface area under P deficiency have been widely reported (Freedeen, Rao & Terry 1989; Jeschke et al. 1996; Nielsen et al. 1998; Rodríguez, Keltjens & Goudriaan 1998a; Mollier & Pellerin 1999; Chiera, Thomas & Rufty 2002). Reductions in leaf area under P starvation could be due to both a lower leaf appearance rate (Etchebest, Plénet & Pellerin 1998; Rodriguez et al. 1998a, b; Rodríguez, Andrade & Goudriaan 1999; Plénet et al. 2000; Chiera et al. 2002) and to a reduced leaf expansion (Etchebest et al. 1998; Rodriguez et al. 1998a; Rodriguez, Andrade & Goudriaan 2000; Plénet et al. 2000; Chiera et al. 2002).

Few studies have focused on processes controlling leaf development in P-starved plants (Radin & Eidenbock 1984; Etchebest et al. 1998; Chiera et al. 2002) and contrasting results have been reported. For instance, the primary effect of P limitation was on the number of cell divisions in soybean (Glycine max [L.] Merr. Chiera et al. 2002) and on cell expansion in cotton (Gossypium hirsutum L. Radin & Eidenbock 1984).

Radin & Eidenbock (1984) concluded that reduced leaf expansion in response to P deficiency was mainly caused by reduced cell expansion due to changes in hydraulic conductance, although other possible causes of growth inhibition such as lower cytokinin content or decreased cell wall elasticity were not excluded. Accordingly, lower cytokinins levels have been found in P-deficient plants (Salama & Wareing 1979; Horgan & Wareing 1980). Furthermore, it has been suggested that P itself could play a key role in the morphogenetic and leaf expansion processes (Freedeen et al. 1989; Rodríguez et al. 1998a). Interestingly, experimental evidence indicates that leaf growth of P-deficient plants would not be limited by carbohydrate supply to growing tissues (Freedeen et al. 1989; Rodríguez et al. 2000).

In monocotyledon leaves, growth is predominately unidirectional and a tissue element is displaced away from the leaf base as a result of production of younger tissue below it (Bernstein, Silk & Läuchli 1993b). Moreover, cell division and tissue expansion are partly overlapping in time and space (Ben-Haj-Salah & Tardieu 1995). The latter processes are restricted to a region known as the growth zone, which is placed in the first centimetres from the leaf insertion point (Schnyder & Nelson 1989; Durand et al. 1995; Tardieu et al. 2000). Consequently, to better understand the physiological mechanisms by which leaf expansion is affected by different environmental stresses it is necessary to study the growing region of the leaf. The appropriate methodology to conduct this kind of studies has been thoroughly described by Erickson (1976), Silk (1984, 1992), Gandar (1980, 1983a, b, c), Schnyder, Nelson & Coutis (1987), Peters & Bernstein (1997) and Tardieu et al. (2000). Whereas many studies have been conducted to analyse the effects of temperature (Ben-Haj-Salah & Tardieu 1995; Tardieu et al. 2000), water deficit and evaporative demand (Ben-Haj-Salah & Tardieu 1997; Tardieu et al. 2000), leaf age (Palmer & Davies 1996) and irradiance (Muller, Reymond & Tardieu 2001) on the growth of maize leaves using this approach, to our knowl-
edge few of them had considered the effects of P deficiency on the growth of maize leaves (Etchebest et al. 1998) and none has been published concerning its effect on cell division rate in the growth zone. The objective of this work was to analyse the spatial distribution of leaf elongation and cell size in leaf 6 of maize, in order to determine if the reduced leaf expansion commonly observed under P stress is associated with a decreased cell production, with a lower final cell size or both.

**MATERIALS AND METHODS**

**Plant material, P treatments and growth conditions**

Seeds of maize (*Zea mays* L. cv Cecilia) were hydrated between sheets of humid tissue paper at 40 °C overnight (6 March 2003) and germinated (7 March 2003) in 1.8 L pots (110 mm upper side length, 80 mm lower side length and 200 mm height) filled with topsoil (0–300 mm depth) of a Haplic Luvisol (FAO-UNESCO 1989) (pH 8.1, 28.1% clay, 59.7% silt, 12.2% sand, 2.65% OM, 14 mg P₂O₅ kg⁻¹ dry soil by Olsen’s method). Micronutrients and macronutrients, other than P, were added in order to provide non-limiting growth conditions. Two soil P levels were studied: ‘low P’ (LP), to which no P fertilizer was added to the soil and ‘control’, to which 113 mg P kg⁻¹ dry soil were added as a solution of NaH₂PO₄·2H₂O. Containers were located in a growth chamber in which photosynthetic photon flux (PPF) at the top of the canopy was around 660 μmol m⁻² s⁻¹, supplied by 400 W OSRAM halogen lamps (VIALOX, NAV-T, SON-T), photoperiod was 16 h, temperature and relative humidity were 24/18 °C and 70/80% (light/dark), respectively. Containers were irrigated daily in order to maintain soil water content at field capacity and received 146 mg N kg⁻¹ dry soil 6 d after seedling emergence (which took place on 11 March 2003). Five, three, two and one plant per container chosen by their overall uniformity, were left 1, 6, 10 and 20 d after seedling emergence, respectively.

Photosynthetically active radiation (PAR) was measured using amorphous silicon cells (Solesm, Palaiseau, France). Air, soil and meristem temperature were measured in spare plants with copper–constantan thermocouples. Air relative humidity was measured with a relative humidity probe (HMP35AC; Vaisala, Helsinki, Finland). All sensors were connected to a datalogger (CR10X; Campbell Scientific, Shepshed, Leics., UK). Measurements that were performed every 10 s and stored every 600 s were used to ensure that target values were maintained. The volumetric water content of the containers was measured daily using a ‘TRIME®-FM2’ TDR (IMKO, Gulf, Ettlingen, Germany).

**Shoot dry weight and P concentration**

In order to determine shoot dry weight and shoot P concentration, six or seven plants per treatment were sampled seven and eight times from 2 to 20 d after seedling emergence, for LP and control treatments, respectively. Plants were dried overnight at 60 °C in a forced-air oven and weighed. Phosphorus concentration was determined by an adaptation of the Malachite green colorimetric technique (Van Veldhoven & Mannaerts 1987) after ashing at 550 °C and dissolving P with nitric acid.

**Elongation rate of leaf 6**

The visible length (*Lₚ*) of leaf 6 was daily measured with a ruler on six plants per treatment to determine leaf elongation rate (*LER*) from 7 d after seedling emergence to the date when leaf 6 was fully expanded (20 and 30 d after seedling emergence for control and LP, respectively). For expanding leaves, leaf length was measured from the tip to the point of junction of leaf borders where the leaf starts to become unfolded and for fully expanded leaves it was measured from the tip to the ligule.

**Spatial analysis of elongation of leaf 6**

Data on the spatial distribution of leaf elongation was obtained by marking the leaf elongating zone with needle holes and evaluating the displacement of the marks during the dark period (Schnyder et al. 1987). Five plants per treatment were marked during the linear phase of leaf elongation in two different dates for each treatment (13 and 14 d, and 17 and 20 d after seedling emergence for control and LP, respectively). The period under study was adjusted in order to obtain a similar total growth of leaf 6 in both P treatments. Twenty-four holes were pierced with fine needles (0.4 mm diameter) inserted through the sheaths of older leaves and the growing region of young leaves. The needles were longitudinally aligned with the centre of the stem and were guided by a piece of plexiglass to ensure a horizontal trajectory. The first hole was close to the scutellar node and neighbouring holes were spaced 5 mm apart.

At the end of the experimental period plants were excised, placed in a box at 4 °C, and transferred to the laboratory. The distances between every hole (*i*) and the base of the leaf were measured using an interactive analysis system (UTHSCSA IMAGE TOOL V 2.00; San Antonio, TX, USA) on digital scanned images of the sheath of a non-growing leaf (initial distance, *L₀*) and of leaf 6 (final distance, *Lₖ*), of each plant after carefully freeing the corresponding leaf from older enclosing leaves. Initial distances should be measured on non-growing leaves as the distances between neighbouring holes could fluctuate because of small errors in needle trajectory. The total length of the leaf 6 was also measured.

The displacement velocities (*vₖ*; mm °Cd⁻¹) where calculated as:

\[
v_i = \frac{\Delta L_i}{\Delta t} \times \alpha
\]

where \(\Delta L_i\) (mm) is the difference between initial (*L₀*, mm) and final distances (*Lₖ*, mm), \(\Delta t\) is the thermal time (°Cd)
of the experimental period, calculated from meristematic temperatures using a base temperature of 10 °C according to Ben-Haj-Salah & Tardieu (1995), and \( \alpha \) is a correction factor estimated as the ratio of mean elongation of non-pierced plants (\( LER_{np} \), mm °Cd\(^{-1}\)) to the elongation of pierced plants (\( LER_p \), mm °Cd\(^{-1}\)). The leaf elongation rate of pierced plants was calculated by summing elongations of all segments after the leaf base. The leaf elongation rate of non-pierced plants was measured during the period of spatial distribution of leaf elongation measurements by making two sequential marks (initial and final) on the blades of leaf 6 in order to indicate their displacement with respect to a fix reference on six non-pierced plants per P treatment. It was assumed that the pierced injury does not affect the spatial distribution of elongation (Schnyder et al. 1987). Then, Eqn 1 can be rewritten as:

\[
v_i = \frac{L_{ei} - L_{si}}{t_i - t_s} \times \frac{LER_{np}}{LER_p}
\]

(2)

Relative elemental growth rates (\( REGR_i \), mm mm °Cd\(^{-1}\)) were calculated as:

\[
REGR = \frac{\delta L}{\delta t}
\]

(3)

In order to be able to compare displacement velocities and \( REGR \)s at similar positions from the leaf base, adjusted displacement velocities (\( V_i \)) for each plant were obtained by fitting Weibull curves as follows:

\[
V_i = a \times \left[ 1 - \exp(-b \times L_i^c) \right]
\]

(4)

and, in agreement with Eqn 3, \( REGR \)s were estimated using the derivative of (Eqn 4):

\[
REGR_i = b \times c \times L_i^{(c-1)} \times (a - V_i)
\]

(5)

### Spatial distribution of epidermal cell density

Cell density (cells mm\(^{-1}\)) was determined in the adaxial epidermis of leaf 6 on the six plants on which leaf elongation had been followed (\( LER_{np} \) determinations). Plants were excised at the same time as those for \( REGR \) analysis. Colourless nail varnish was spread on the adaxial epidermis of leaf 6 in order to indicate their displacement with respect to a fix reference on six non-pierced plants per P treatment. It was assumed that the pierced injury does not affect the spatial distribution of elongation (Schnyder et al. 1987). Then, Eqn 1 can be rewritten as:

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v_i = \frac{L_{ei} - L_{si}}{t_i - t_s} \times \frac{LER_{np}}{LER_p}
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(5)

**Cell division rate, cell flux and duration of cell displacement in the growth zone**

Tissue elements within the growth zone could be viewed as being displaced or moving through it and, assuming a continuous smoothly varying medium (e.g. the linear phase of leaf elongation), methods of fluid dynamics can be used to analyse the leaf growth (Silk 1984). In this way, the cell production rate can be inferred from the spatial distributions of cell density and of the local elongation rate (Gandar 1980; Silk 1992; Tardieu et al. 2000). The method is based on the calculation of the flux through a small element of the leaf (e.g. the number of cells that cross a 1-mm-wide leaf strip, located at a distance \( L_i \) from the leaf base and perpendicular to the midrib, in one °Cd). Based on the principle of mass balance and according to Silk (1992) the number of new cells that appear in the element (cell production rate, \( d_i \), cell mm\(^{-1}\) °Cd\(^{-1}\)) is calculated as:

\[
d_i = \frac{\delta \rho}{\delta t} \times \rho \times REGR + \frac{\delta \rho}{\delta L_i} \times v_i
\]

(6)

where \( \frac{\delta \rho}{\delta t} \) (cell mm\(^{-1}\) °Cd\(^{-1}\)) is the change in cell number in segment \( i \), \( \rho \) is the cell density per unit of leaf length (cell mm\(^{-1}\)), reciprocal of cell length in segment \( i \), \( v_i \) (mm °Cd\(^{-1}\)), integration of \( REGR \), from the base of the leaf to the segment \( i \) is the local displacement velocity and \( \frac{\delta \rho}{\delta L_i} \) (cell mm\(^{-1}\)) is the gradient in cell density.

The first term (local change) could be set to zero, assuming that profiles of cell length at the beginning and end of the measuring period did not change (MacAdam, Volenece & Nelson 1989; Ben-Haj-Salah & Tardieu 1995; Tardieu et al. 2000). The second and third terms could be calculated only in cases when \( LER \) and \( REGR \) are constant over the measuring period.

Cell division rates (\( D_i \), cell cell\(^{-1}\) °Cd\(^{-1}\)) were calculated from \( d_i \) (cell mm\(^{-1}\) °Cd\(^{-1}\)) and cell density (\( \rho_i \), cell mm\(^{-1}\)) using:

\[
D_i = \frac{d_i}{\rho_i}
\]

(7)

The local cell flux in segment \( i \) (\( f_i \), cells °Cd\(^{-1}\)) was calculated as the integral of production rates from the leaf base to segment \( i \). This integral is the product of cell density (\( \rho_i \), cell mm\(^{-1}\)) and local velocity (\( v_i \), mm °Cd\(^{-1}\)):

\[
f_i = \rho_i v_i
\]

(8)

The time (\( t_i \), °Cd) needed for a material point (cell) to move from the proximal point to the distal limit of segment \( i \) was calculated as the ratio of the length of the segment (\( \delta L_i \), mm) to the local velocity (\( v_i \), mm °Cd\(^{-1}\)):

\[
t_i = \frac{\delta L_i}{v_i}
\]

(9)

The total time needed for a material point to move from its present position to the distal end of the growth zone was calculated as the sum of \( t_i \) from its actual position to the distal limit of the growth zone (Schnyder et al. 1990). It is therefore, presented on a negative scale.
Statistical analyses

A completely randomized design with a single factor (P treatment) was used. For most of the measured variables, the analyses of variance were carried out per date or per position from leaf base. Analyses of variance (ANOVA) were performed using SAS GLM procedure (SAS Institute, Cary, NC, USA). Means were separated using the LSD at the $P = 0.05$ significance level. Curves were fitted using the Gauss–Newton algorithm subroutine of the procedure NONLIN of SYSTAT 9.01 for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

Overall plant development and P content

Shoot dry weight in LP plants began to significantly deviate from that of control plants at around 130 °Cd after seedling emergence and was approximately 33% of control plants at the time when leaf 6 was completely expanded (i.e. around 260 and 380 °Cd for control and LP, respectively; Fig. 1a). Final leaf length was significantly diminished by P deficiency from leaf number 4 onwards (data not shown).

Shoot P concentration as a function of thermal time showed a similar pattern in both P treatments (i.e. it abruptly decreased until approximately 110 °Cd and tended to stabilize afterwards; Fig. 1b). Significant differences in P concentration between control and LP plants were found from the second sampling date (approximately 57 °Cd after seedling emergence). Shoot P concentration was reduced by around 36% in LP plants compared with the control plants by the time when leaf 6 was completely expanded (Fig. 1b).

Elongation rate of leaf 6

Limited P availability reduced LER of leaf 6 by approximately 63% during the linear phase of leaf elongation (Fig. 1c) compared with the control treatment. It can be seen in Fig. 1c that LER of control leaves at the end of the linear phase of elongation (around 250 °Cd) abruptly fell to values similar to those shown by LP leaves. In LP leaves this reduction was not as steep and it took place at around 400 °Cd.

Spatial analysis of leaf elongation

In this experiment, for each P treatment, two measurement periods were carried out during the linear phase of elongation of leaf 6 when LER values remained relatively constant in time (Fig. 1c). It has been shown that in steady growth, as in this case, the displacement velocity curve does not change with time (Silk 1992). Therefore, the results of both periods corresponding to the same P treatment were analysed in conjunction.

Average total leaf length of leaf 6 used for spatial analysis of leaf elongation for control and LP treatments were 421.6 ± 69.4 and 290.9 ± 41.0 mm, respectively; which included 4.2 ± 1.0 and 3.1 ± 0.8 mm of sheath for the same treatments. Total leaf lengths were nearly 70% of the respective final leaf blade lengths (control = 617.3 mm and LP = 405.7 mm, SE = 16.7 mm), which means that measurements were taken at approximately the same phenological stage.

As expected, the displacement velocity increased with distance from the leaf base (Fig. 2a) until the end of the growth zone, defined for the purpose of this article as the

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position in which adjusted displacement velocity reached 95% of its maximum (control = 100.3 mm mm⁻¹ Cd⁻¹ and LP = 43.8 mm mm⁻¹ Cd⁻¹, SE = 3.6 mm mm⁻¹ Cd⁻¹). The determination coefficient for all velocity adjusted curves was higher than 0.93. The small size of the SEs of the 10 replicate observations at each position within the growth zone indicates the high degree of reproducibility found among leaves.

Interestingly, the basal part of the growth zone was less affected by P deficiency. Consequently, differences between P treatments started to be significant at 32.5 mm from leaf base and remained significant for every position thereafter (Fig. 2b).

Spatial distributions of REGR values for both P treatments are shown in Fig. 2c. Phosphorus deficiency significantly diminished the maximum REGR (control = 0.101 mm mm⁻¹ Cd⁻¹ and LP = 0.090 mm mm⁻¹ Cd⁻¹, SE = 0.004 mm mm⁻¹ Cd⁻¹) and shifted its location closer to the leaf base (control = 32.6 mm and LP = 18.0 mm, SE = 1.8 mm), in our experiment by 11 and 45%, respectively.

Spatial distribution of epidermal cell size and duration of cell displacement in the growth zone

Adaxial epidermal cells were significantly longer in LP than in control leaves for all positions from the leaf base except at 97.5 mm (Fig. 3a). This means that mature epidermal cell lengths were similar between P treatments. Similarly, differences in epidermal cell surface area in the mature zone were not significant, although control cells tended to be shorter.
slightly larger than LP (e.g. control 3146 ± 152 μm² and LP 3060 ± 137 μm²).

The curves of cell displacement in the growth zone indicate that, under our experimental conditions, it took around 100 °Cd for a cell situated at 2.5 mm from the leaf base to reach the limit of the growth zone and only about 20 °Cd to reach the mature length once it had approximately 20% of it (Fig. 3b). Interestingly, when cell length was related to thermal time necessary to reach the end of the growth zone, no significant differences were found between curves, indicating that P deficiency did not affect the period of cell elongation (Fig. 3c).

Cell production and cell flux

The average length of the cell division zone (defined as the position when cell production rate first reached zero) was decreased by 21% in LP leaves (control = 27.1 mm and LP = 21.3 mm, SE = 1.5 mm, Fig. 4a). Consequently, cell division activity continued until the \( LER \) values were around their maximum and well beyond the location where cell length started to increase (Fig. 3a). Although maximum cell production rate was similar between P treatments (control = 2.99 cell mm\(^{-1} \)°Cd\(^{-1} \) and LP = 2.36 cell mm\(^{-1} \)°Cd\(^{-1} \), SE = 0.30 cell mm\(^{-1} \)°Cd\(^{-1} \)); the decrease of cell production rate with distance from the base was more pronounced in LP, so significant differences were found from 12.5 mm from the leaf base. The cell division rate, basically reflected cell production patterns and similarly, no significant differences were found between treatments in the maximum values achieved (control = 0.055 cell cell\(^{-1} \)°Cd\(^{-1} \) and LP = 0.050 cell cell\(^{-1} \)°Cd\(^{-1} \), SE = 0.004 cell cell\(^{-1} \)°Cd\(^{-1} \)). The slight but significant difference in cell size between treatments at the base of the growth zone could also have contributed to the lower cell production in LP in comparison with the control leaves. Even when cell division rate was not different between treatments for some extent at the base of the division zone (i.e. less than 12.5 mm), the fact that slightly fewer cells were situated at each position determined that a lower number of cells could be produced per unit length.

Because of the differences in cell production rate and in the length of the cell division zone, total cell production (i.e. the integration of the cell production rate along the division zone) was approximately twice as much in control than in LP. Consequently, cell flux was significantly higher in control than in LP leaves from 17.5 mm from the leaf base (Fig. 4b).

Since the time required for a cell to leave the growth zone was similar in both P treatments (Fig. 3b), cell flux in the mature zone (i.e. outside the growth zone; Fig. 4b) essentially reflected the higher cell production associated with a longer cell division zone with higher cell production rates in control compared to LP. Furthermore, since this flux in the mature zone could also be calculated as the ratio of \( LER \) to mature cell length, and the latter was similar in both P treatments, the difference in final cell fluxes between treatments was proportional to the difference in \( LERs \).

DISCUSSION

As expected, shoot dry weight, shoot P concentration and \( LER \) values of leaf 6 were all reduced by P deficiency (Fig. 1). Although shoot P concentration profiles are in agreement with those found by Plénet et al. (2000) in a maize field experiment; the values measured in the present experiment were lower even for control plants. This disparity could be related to differences in experimental conditions, and in particular, to the limited soil volume available per plant in our experiment.

The growth zone of leaf 6 was decreased by around 56% in LP compared with the control plants (Fig. 2). As it has been argued above, its length in control plants was longer than previously found in maize (Ben-Haj-Salah & Tardieu 1995, 1997; Tardieu, Granier & Muller 1999; Tardieu et al. 2000; Etchebest et al. 1998). One of the possible causes of this discrepancy, besides the differences among the cultivars studied, could have been a lower irradiance in the present experiment. Accordingly, it has been reported that the growth zone of tall fescue (Festuca arundinacea Schreb.) leaves were longer at lower irradiance (Schnyder & Nelson 1989).
In our experiment, no differences in the displacement velocity curves between P treatments were found in the basal part of the growth zone (Fig. 2b). A similar pattern of divergences between the velocity profiles of treated and control plants (Fig. 2b) were observed in leaves of sorghum (Sorghum bicolor [L.] Moench) subjected to NaCl stress (Bernstein et al. 1993b). However, in some experiments of abiotic stresses differences between velocity profiles in leaves started to be apparent closer to the base of the growth zone (e.g. Bernstein, Läuchli & Silk 1993a; for salinity stress in sorghum; Snir & Neumann 1997 for mineral nutrient deficiency in maize).

A reduction of maximum REGR and a change of its location closer to the leaf base were observed here in response to P deficiency (Fig. 2c). Similarly, these responses have been observed earlier in many studies in which plants were subjected to stresses that reduced their LER values. For example, as a result of a salinity stress in sorghum (Bernstein et al. 1993a, b) and of a progressive water deficit in tall fescue (Durand et al. 1995) and maize (Tardieu et al. 2000). Conversely, it has been found that a constant water deficit under low evaporative demand reduced REGRs at all positions in the growth zone of leaf 6 of maize, but it did not move the location of the maximum values (Tardieu et al. 2000).

Interestingly, the cell division zone was shorter and cells within it were longer in LP than in control leaves. This means that there was a reduced total number of dividing cells under P deficiency. Shorter cell division zones were also found in leaf 6 of maize subjected to water deficits (Tardieu et al. 2000); however, water deficit also lowered maximum cell division rates, which was not the case for P deficiency in this experiment (Fig. 4a).

There are several examples in the literature in which an applied stress reduced leaf growth and cell production proportionally, without changing the elongation of individual cells (Volenc & Nelson 1983; MacAdam et al. 1989 and Gastal & Nelson 1994 for N deficiency in tall fescue); whereas in others the leaf growth inhibition was associated with reductions in cell size (Neumann, Van Volkenburgh & Cleland 1988 for salinity stress in Phaseolus vulgaris L.; MacDonald 1989 and Palmer et al. 1996 for N deficiency in Salix viminalis L. and Helianthus annuus L., respectively; Jacob & Lawlor 1991 for P deficiency in H. annuus L., Z. mays L. and Triticum aestivum L.); or in which both responses were observed depending of the degree of development of the leaf when the stress was applied (Lecoeur, Wery & Sinclair 1996 for water deficit in Pisum sativum L.). Our results are in agreement with the former, as no significant differences in the final adaxial epidermal cell length between P treatments were found (Fig. 3a), the reduction in leaf growth under P deficiency was caused by a reduced cell production (Fig. 4a). Specifically for the case of P a similar response was observed by Chiera et al. (2002) in epidermal cells of soybean leaves, although they found that spongy mesophyll cells were smaller under P deficiency.

In agreement with our findings it has been observed that epidermal cells of growing leaves of tall fescue receiving low N were longer than those receiving high N for some extent along the basal part of the growth zone but they had a similar mature length (Volenc & Nelson 1983). However, in this grass species the epidermal cells subjected to high-N treatment elongated for a longer period to reach the mature length (MacAdam et al. 1989), which was not the case for control leaves here (Fig. 3b). For instance, in Fig. 3c it is possible to observe the similitude between the curves of cell size versus thermal time needed to leave the growth zone of both P treatments. Similarly, no differences in the duration of cell elongation were found by Frike & Flowers (1998) in barley (Hordeum vulgare L.) plants subjected to different availability of N. It should be noticed, however, that after a certain considered period a cell located at the same initial position from the leaf base was displaced further away in control than in LP leaves (Fig. 3b) because of a longer growth zone, and consequently, a greater number of elongating cells undergoing a similar rate of expansion at the same time in the control treatment.

Interestingly, shorter periods of cell elongation than those measured here have been reported for maize epidermal cells (Tardieu et al. 1999, 2000). It is not clear if this discrepancy was due to genetic or to environmental differences among experiments.

Our results contrast with those reported by Snir & Neumann (1997) on early inhibition of leaf growth caused by nutrient deficiency of hydroponically grown maize seedlings. They did not detect differences between treatments in the length of the growth zone, they found decreased REGR values in the basal part but not in the apical region of the growth zone, and they observed inhibition of leaf cell expansion (i.e. reduction in mature cell length). We suggest that the differences among the responses reported above and our results might have been due to the fact that in our case, time spatial analyses of leaf elongation were performed on a leaf that had started its development almost simultaneously with treatment imposition (e.g. leaf 6 measured 0.5–1.0 mm at approximately 30 °C after seedling emergence) whereas the authors above studied leaf 1, which is a leaf that is already preformed in the embryo (Tardieu et al. 1999). Moreover, here the only deficient nutrient in the rooting media was P and it was also present in the LP treatment. This experimental difference may partially explain why leaf 1 was smaller under nutrient deficiency in their experiment, but not in ours (control = 46.4 mm, LP = 46.2 mm, SE = 0.6 mm).

It would be interesting to investigate if P itself, as a major plant nutrient required for biosynthesis of macromolecules (e.g. nucleic acids, phospholipids), or changes in hormonal concentrations such as increased abscisic acid (Jeschke et al. 1997) or reduced cytokinins (Salama & Wareing 1979), among others, are involved in the lower cell production rates observed in LP leaves. With respect to the former hypothesis, Sano et al. (1999) have suggested that P starvation would cause abnormal cell plate formation in tobacco (Nicotiana tabacum L.) cells. They also have observed that the addition of phosphate to phosphate-starved cells induced them to re-enter the cell cycle (i.e. cells semi-syn-
chronically progressed through S-phase into mitosis) and that their physiological conditions in the static state during P starvation, as well as the signalling to re-enter the cell cycle, differed from those of axin-starved cells. Moreover, a gene (phi-1), that might play a role in the phosphorylation of certain substrates, was rapidly induced (Sano et al. 1999) and a MAP (‘mitogen-activated-protein’) kinase was activated upon phosphate-induced cell cycle re-entry of phosphate-starved tobacco cells (Wilson et al. 1998). Although, it is known that MAP kinases are components of the transduction pathways of different growth factors, hormones and other mitogenic signals that induce mammalian cells to re-enter the cell cycle and proliferate, their role in the control of the cell cycle in plants is less clear (Wilson et al. 1998).

In summary, our results show that the lower LER measured in leaf 6 of P-deficient maize plants compared with control plants was associated with a lower cell production. This decreased cell production was determined by a shorter zone of cell division with lower cell production rates along most of its length in LP treatment compared with control. It has been also observed that epidermal cells have apparently a certain genetically determined elongation capacity (i.e. genetically determined mature cell length) that was not affected by P deficiency, so the growth zone was longer in control than in LP leaves because of a greater number of cells moving through it. Further experiments should try to elucidate whether the effect of P itself or changes in hormonal concentrations are involved in the lower cell production of P-deficient maize leaves.

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