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Nitrogen deprivation induces changes in the leaf elongation zone of maize seedlings

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Abstract

The influence of nitrogen deprivation on leaf development and the biomechanics of leaf growth were studied using maize (*Zea mays* L.) seedlings grown under low irradiance. Although the nitrogen deprivation had no significant effect on photosynthesis, the leaf length, the leaf area, and the total assimilation area of plants decreased. The mature size of the epidermal cells was not altered, while the cells of nitrogen-deprived plants reached their final length closer to the leaf base than the epidermal cells of control plants. Decreases in the length of the growing zone (from 50 to 30 mm) and in the maximum value of relative elemental growth rate (from 0.08 to 0.06 mm mm^{-1} h^{-1}) were observed in the nitrogen deprived plants. The maximal value of growth velocity in the control treatment was higher along the elongation zone, except for the basal 20 mm, where there was no significant difference between the control and the N-deprived plants. The net deposition rates of water and dry matter were also affected by nitrogen deprivation: the values of these features decreased and the spatial position of the maximum of the deposition rates shifted towards the leaf base.

Additional key words: epidermal cell size, maize leaf growth, photosynthesis, relative elemental growth rate, *Zea mays*.

Introduction

On various levels of its organization, the development and growth of a plant is ultimately dependent on photosynthesis. In most cases, the amount and finally the total area of the photosynthetic tissue determine the productivity of the plants (Kriedeman 1986, Osmond 1987). Although the interrelation of the photosynthesis and growth has been widely studied (Evans 1976, Ceulemans and Impens 1983, Bowler and Press 1996), it is still difficult to interpret the effect of photosynthesis on the growth because of our incomplete understanding of the mechanism controlling carbon allocation and redistribution.

As one of the most important nutrient elements, nitrogen affects the growth of plants (Radin and Boyer 1982, MacAdam *et al.* 1989, Palmer *et al.* 1996). Nitrogen deprivation may affect the growth indirectly by changes in cell pressure potential (Radin and Boyer 1982, Chapin *et al.* 1988, Taylor *et al.* 1993), cell wall properties (Taylor *et al.* 1993, McDonald and Davies 1996), contents of phytohormones, or secondary signal metabolites (Kuiper 1988, Samuelson *et al.* 1992). Nitrogen supply may also modify the division and expansion of epidermal and mesophyll cells (Trewavas 1985, MacAdam *et al.* 1989, Dodd and Davies 1994).

Leaves of grasses seem to be the best suitable models for the study of basic processes involved in the leaf expansion since the zone of cell division and elongation in the leaf blade is limited to the basal section of the leaf (Volenec and Nelson 1982, Silk 1984, Schnyder *et al.* 1987, Morris and Silk 1992). In these plants, division and elongation of cells are predominantly unidirectional leading to creation of parallel files of cells (MacAdam *et al.* 1989). With distance from the meristem a single cell in this file of cells passes from one developmental stage

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Abbreviations: REGR - relative elemental growth rate; LA - leaf area; NDR - net deposition rate.

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to another, displaced from it original site by divisions and growth of younger cells (Silk 1984, Schnyder and Nelson 1987, Schnyder *et al.* 1987).

Nitrogen deprivation depresses the photosynthesis through decrease of availability of Rubisco and thus reduces amounts of assimilates involved in the control of growth of newly emerging leaves (Gastal and Nelson 1994). However, the decrease of photosynthesis is light-dependent and expected to appear primarily at high irradiance. We hypothesized that under low irradiance the N deprived plants could utilize similar amount of assimilates per leaf area as the unstressed plants. The source is equal, then other factors, like redistribution of assimilates, non-photosynthetic production of sugars, could seriously affect the process of growth, which functions as an active sink of assimilates (Schnyder and Nelson 1987, Schnyder *et al.* 1987).

Materials and methods

Plants and growth conditions: Maize (*Zea mays* L. cv. Maya) was used. Seeds were soaked for 6 h in distilled water and germinated for 48 h on moist blotting paper in dark, at temperature of 22 °C. The seedlings were planted into pots of 10 cm diameter filled with moist perlite. Four seedlings were planted in each pot at a depth of 3 - 4 cm and grown in a growth chamber (*E7/2, Conviron*, Pembina, USA): 14/10 h photoperiod, day/night temperatures 24/18 °C, relative humidity *ca.* 60 % and irradiance of 200 μmol m^{-2} s^{-1} (white cool tubes - *F48T12*, *Sylvania*, Drummondville, Canada). The seedlings were watered with full nutrient solution until the second leaf emerged. Then 20 pots containing plants of the same size were chosen and were divided into two groups. The first group (+N) received complete nutrient solution, consisted of KNO_3 (4 μmol), $Ca(NO_3)_2$ (3 μmol), $MgSO_4 . 7\ H_2O$ (3 μmol), $NaH_2PO_4 . 2\ H_2O$ (1.5 μmol), where nitrogen was added in nitrate-N form, while the second group (-N) was supplied with nitrogen-free solution consisted of $MgSO_4 . 7\ H_2O$ (3 μmol), $Na_2HPO_4 . 12\ H_2O$ (1.5 μmol), K_2SO_4 (4 μM), $CaCl_2 . 2\ H_2O$ (4 μmol).

The treatments were performed for 10 d and experiments were repeated four times. In the survey, the results of a representative experiment are presented.

Measurement of leaf photosynthesis: Photosynthetic rate of 1st, 2nd and 3rd leaves was measured at the end of the experimental period. Measurements were performed between 9:00 and 11:00 in the growth chamber using *LCA2* photosynthetic system (*ADC*, Hoddesdon, UK.).

Growth measurements: From the appearance of the second leaf, 15 seedlings per treatment were measured from the tip of the coleoptile to the end of the leaf blade daily. Foil used for measurements had millimetre grids that allowed determining the leaf length with precision up to 0.5 mm.

Plants were harvested at the end of the 10-d treatment and the area of every leaf was measured by a scanner and a computer program *Areascope v1.5* (Mészáros *et al.* 1993). The data for the leaf length and related leaf areas, measured on 15 plants from each treatment, were used to establish a linear regression in order to make the indirect estimation of the leaf area during treatments.

Water content, dry matter and cell size were measured in the fourth leaves.

Water content and dry mass measurements: Tissue samples from 8 - 10 plants were collected along the fourth leaf with parallel razor blades, producing 3-mm long leaf segments into Eppendorf vials, and were kept on ice. After fresh masses were determined, the samples were dried at 105 °C in an oven for 48 h. The water content was calculated as the difference of fresh and dry mass.

Measurement of epidermal cell size: Leaf segments (3 mm) were cleared of pigments by boiling them in methanol. The samples were kept under absolute ethanol-glycerine-distilled water (1:1:1). The length of epidermal cells on the abaxial side of leaf segments was measured directly using a light microscope (*CX20, Olympus*, Tokyo, Japan). On five tissue samples from the same leaf segment, 200 cell length measurements were performed.

Growth analysis: For the determination of the relative elemental growth rate (REGR), as well as of the cell flow rate from the data on cell size profile in the elongation zone, the method described in Silk *et al.* (1989) and Morris and Silk (1992) was used:

$$\mathrm{REGR} = \delta v/\delta z = f \times \delta l/\delta z,$$

where REGR is the relative elemental growth rate or strain rate of a cell at spatial position z, l is the local cell length at the same spatial position, while v is the local velocity, *f* is cell flux. Cell flux, that is the number of cells passing the given point *z* per unit time, could be evaluated as ratio of local cell velocity and local cell length in the same spatial position, which in certain conditions at the base of the elongation zone could be substituted for by the ratio of organ extension rate (LER - leaf elongation rate) to the mature cell length

$$\mathrm{REGR} = \mathrm{LER}/l_m \times \delta l/\delta z,$$

where LER is the leaf elongation rate, l_m is the mature cell length (Sharp *et al.* 1988, Moris and Silk 1992, Silk

1992). According to Silk (1992), growth velocity (u) (also known as displacement velocity) is the first integration of the equations of the REGR, while as a result of another integration the growth trajectories could be obtained.

Local net deposition rates (NDR) of water and dry matter in the elongation zone of the fourth leaf of maize were calculated using 3-mm-long segments of the elongation zone and the one-dimensional version of the continuity equation described by Silk (1984),

$$NDR = \delta p/\delta t + u \times \delta p/\delta x + p \times \delta u/\delta x,$$

where p is the density of water or dry matter (water and dry matter content), u is growth velocity and t is time.

Results

Net photosynthesis rate of leaves: P_N of the third leaf of -N seedlings was slightly lower than the P_N of the 3rd leaf of control plants, but the difference was not significant (Table 1). Net photosynthetic rate of the leaves at saturated irradiance (1400 μmol m^{-2} s^{-1}), P_{max}, for +N and -N treatments was 12.24 and 8.93 μmol [CO_2] m^{-2} s^{-1}, respectively. P_N at growth conditions as compared to P_{max} was lower by 54 and 48 % for control and nitrogen deprived treatments, respectively.

Table 1. Net photosynthetic rate, P_N [μmol [CO_2] m^{-2} s^{-1}] of maize seedlings at the end of the experimental period at growth irradiance of 200 μmol m^{-2} s^{-1}. Means of 10 measurements ± SE. Differences were not significant according to unpaired *t*-test.

	+N	-N
1st leaf	1.95 ± 0.39	2.08 ± 0.71
2nd leaf	3.37 ± 0.37	3.81 ± 0.15
3rd leaf	5.72 ± 0.92	4.64 ± 0.61

Leaf length and leaf area: After 10-d treatment, the first and second leaves of the +N and -N maize seedlings showed no significant differences (Table 2). However, the leaf growth of 3rd and 4th leaves was significantly reduced under -N, and these leaves were 42 and 57 % shorter and the area of the leaves was 50 and 70 % smaller as compared to the +N treatment (Table 2).

As a result, the total leaf area of seedlings progressively decreased (Fig. 1). The reduction in the total leaf area became more apparent on the 4th and 8th days of the experiment, when the 3rd and 4th leaves appeared. At the end of the experiment the total leaf area of the -N treatment was 27.3 % lower as compared to the +N treatment (Fig. 1, Table 2).

Spatial distribution of cell size: The cell length increased with the distance from the base of the leaf towards the end of the elongation zone (Fig. 2). Differences in nitrogen supply had no significant effect on the mature cell size. The length of a mature cell in the epidermis of leaves was 148 ± 35 μm and 155 ± 25 μm in the +N and -N plants, respectively (Fig. 2). The nitrogen deprivation, however, affected the spatial profile of cell length through the elongation zone. Basipetally to the position at which mature length was attained, cells were longer in the leaves from -N seedlings (Fig. 2). The inflection point of the curve describing the distribution of the epidermal cell size was 19.2 and 25.7 mm from the base of the leaf for the -N and +N treatments, respectively. While the mature cell sizes in +N plants were achieved at the distance of 70 - 80 mm from the leaf base, in -N plants the cells reached their maximal length at a 50 - 60 mm distance from the base of the leaf. These changes became more obvious when relative cell length was calculated as the ratio of treated to control cell length (Fig. 2*B*). At the base of the leaf in -N plants this ratio increased and showed larger cell lengths up to 60 %, than in the case of the +N plants (Fig 2*B*).

The relative elemental growth rate (REGR) under growth optimal conditions had approximately a bell-

Table 2. Leaf length and area of maize seedlings treated with optimal (+N) and sub-optimal (-N) amounts of nitrogen for 10 d. Means of 10 measurements ± SE.

Leaves	Length [cm] +N	-N	Area [cm^2] +N	-N
1st leaf	5.70 ± 0.15	6.75 ± 0.81	5.12 ± 0.26	4.19 ± 0.49
2nd leaf	12.80 ± 1.31	15.95 ± 1.04	11.27 ± 0.80	13.50 ± 1.17
3rd leaf	24.23 ± 0.68	14.15 ± 1.32	24.46 ± 1.16	12.43 ± 1.49
4th leaf	14.10 ± 1.41	6.12 ± 0.74	14.13 ± 1.92	4.27 ± 0.16

shaped profile (Fig. 3*A*). The deprivation of nitrogen affected both the maximum value of the REGR (decreased from 0.08 to 0.06 mm mm^{-1} h^{-1}) and the spatial position of the maximum, shifting it towards the base of the leaf blade (the REGR reached its maximum at 20 - 25 mm in the plants supplied with nitrogen and at 15 mm, when nitrogen was deprived (Fig. 3*A*).

The growth velocity increased with the distance from the base of the leaf blade and up to the distal end of the elongation zone, where the velocity became constant and velocity at the end of the elongation zone of the 4th leaf in equalled the leaf elongation rate (Fig. 3*B*). The growth -N plants was lower by 39 % than in +N plants. However, in the basal 20 mm of the leaf blade the growth velocity did not differ in control and stressed plants, and was even slightly higher in the later ones.

Distribution of water and dry matter in the elongation zone: The spatial distribution of dry matter (Fig. 4*A*) and water content (Fig. 4*B*) in the fourth leaves of the maize changed significantly due to nitrogen deprivation: the pattern of the change was similar to the change in the REGR (Fig. 3*A*). In leaves of control seedlings, the dry matter content per 3 mm per section varied from 0.08 to 0.14 mg and showed characteristically high values around 20 and 80 mm from the leaf base. In the leaves of the -N plants, the spatial distribution of the dry matter changed and sections with high dry matter content were shifted closer to the leaf base by 20 mm. The highest dry matter content in the leaves of -N plants was observed in the base peak and was 0.19 mg per 3-mm section. Due to the shift of the dry matter distribution pattern, there were places where the dry matter of the control plants exceeded the values from the -N treatment, and *vice versa.*

In the leaves of +N plants, the water content of tissues per 3 mm section increased from 0.8 mg at the base of the leaf to 1.6 mg at the end of the sampling region (Fig. 4*B*). Under nitrogen deprivation, the water content of the leaf was higher at the base of the leaf and exceeded that of the +N plants by 40 % (Fig. 4*B*). From the base of the leaf, the water content of tissues in the -N plants decreased, and between 20 and 60 mm from the base it was nearly the same as in the +N treatment. Further away from this part of the leaf, until the end of the sampling zone, the water content was significantly lower than in -N plants of +N plants (Fig. 4*B*).

Dry matter and water deposition: The net deposition rate (NDR) of dry matter showed two peaks along the

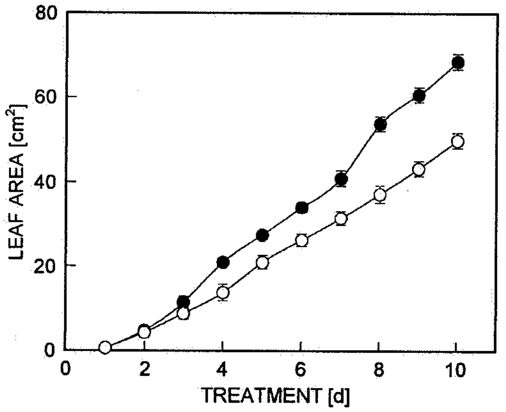

Fig. 1. Change in leaf area of plants as a result of nitrogen deprivation. Means ± SE ($n = 15$).

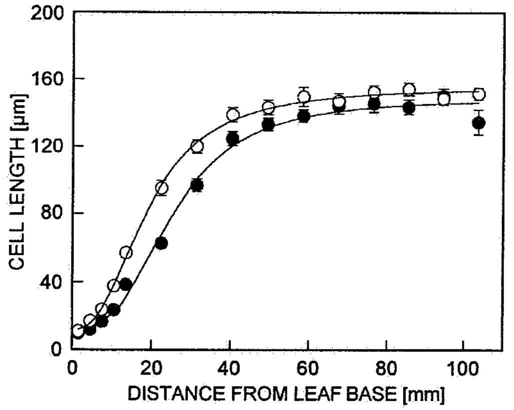

Fig. 2. Effect of N-deprivation on the spatial distribution of cell length in the basal 100 mm of the fourth leaf of maize. Means ± SE ($n = 200$). The curves are the result of fitting a sigmoid curve to the data obtained from cell length measurements.

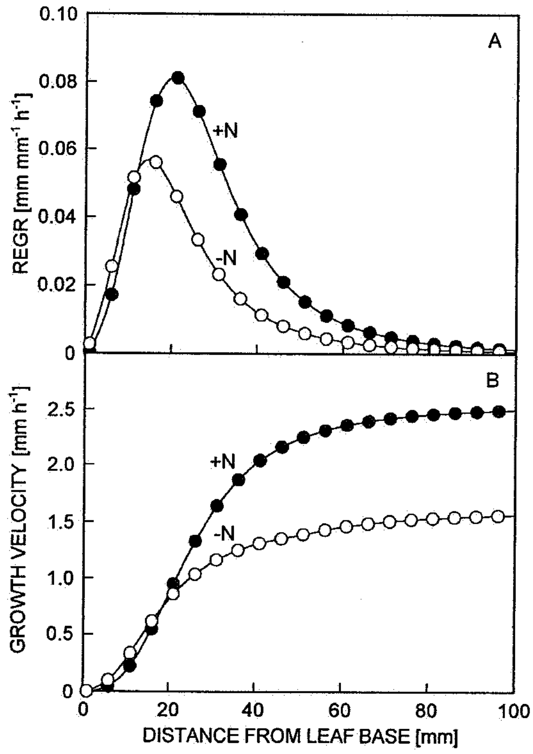

Fig. 3. Change of relative elemental growth rate (REGR, *A*) and the growth velocity (*B*) as affected by N-deprivation in the basal 50 mm of the fourth leaf of the maize.

sampling zone of fourth leaf (Fig. 5*A*). The first peak appeared at the locations of the most rapid growth, at 20 - 25 mm from the base of the leaf in +N plants and 10 mm from the base of the leaf in -N plants. The control seedlings displayed another dry matter deposition rate peak after a short region of decrease at around 60 - 70 mm from the base of the leaf (Fig. 5*A*), while there was a not so obvious peak for the -N plants at the region around 50 mm from the base of the leaf. The magnitude of the highest value was lower in the -N plants, and the location of peaks in -N plants was shifted towards the leaf base, as compared to the control ones. The similarity between the profile of REGR (Fig. 3*A*) and the first peak of NDR in +N and -N plants (Fig. 5*A*) should be noticed.

The deprivation of nitrogen has two effects on the spatial change of the NDR of water within the leaf (Fig. 5*B*). Firstly, it decreased the magnitude of the maximum value of the deposition rate (from 0.3 to 0.2 mg mm^{-1} h^{-1}) and, secondly, shifted the position of the maximum value towards the base of the leaf blade. Rates of water deposition in the -N plants were not lower along the whole elongation zone as compared to the +N plants (Fig. 5*B*); moreover, in the basal 20 mm of the sampling zone the net rate of water deposition was higher in the -N plants as compared to the +N ones. Starting from 70 mm from the base of the leaf the net deposition rate of water in the +N treatment was higher ($P < 0.01$). Quite interestingly, peaks in both control and stressed leaves coincided with the regions of rapid longitudinal cell growth (Fig. 5*A*).

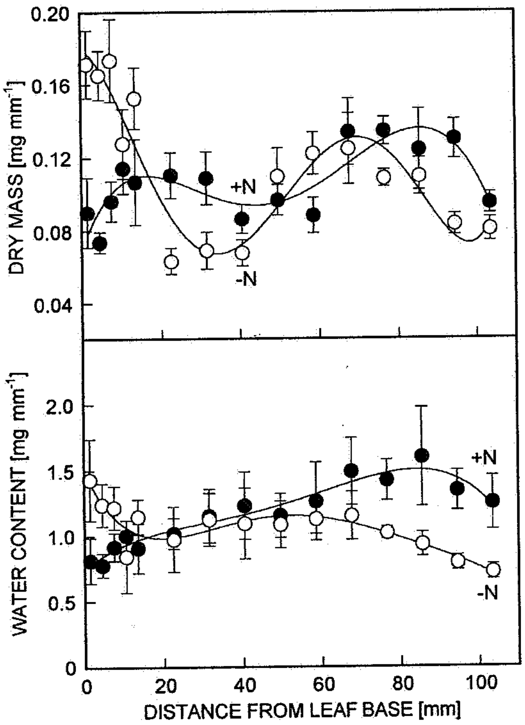

Fig. 4. Spatial distribution of (*A*) dry mass and (*B*) water content through the basal 100 mm of the fourth leaf of maize as affected by N-deprivation. Means ± SE ($n = 6$).

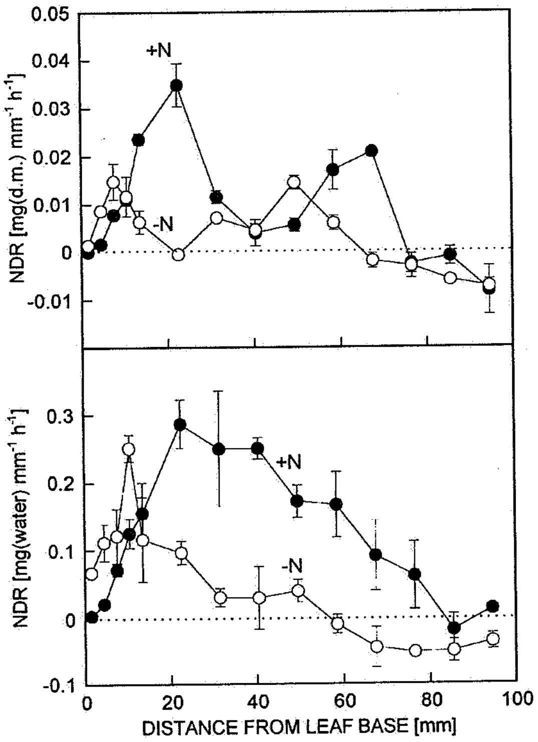

Fig. 5. Changes of net deposition rate (NDR) of (*A*) dry mass and (*B*) water in the basal 100 mm of the fourth leaf of maize as affected by N-deprivation. Means ± SE ($n = 6$).

Discussion

The study provides information on the growth mechanism in the leaves of maize seedlings developing under nitrogen deprivation and grown under low irradiance. A rapid decrease in leaf area appeared shortly after the deprivation of nitrogen from the nutrient solution primarily as a result of the reduced elongation zone of leaves, which correlates with other results on wheat (Osman *et al.* 1977), birch (Ingestad and Lund 1979), sunflower (Radin and Boyer 1982, Palmer *et al.* 1996), tall fescue (MacAdam and Nelson 1987, MacAdam *et al.* 1989), barley and tomato (Chapin *et al.* 1988), and small willow (McDonald 1989).

The 1st and 2nd leaves that emerged before the nitrogen deprivation were not affected (Tables 1 and 2). On the subsequent leaves the effect of nitrogen deprivation occurred progressively: the final leaf length and area of the -N plants was only 42 and 47 % for the third leaf as compared to the +N plants, and reached 57 and 70 % for the fourth leaf. These might suggest that the translocation of nitrogen from older leaves can moderate the reduction of the N from the nutrient solution, but cannot diminish the effect of nitrogen deprivation. The decrease in the total leaf area of the seedlings became apparent on the 4th day due to the appearance of younger leaves (Fig. 1).

The nitrogen deprivation affected the elongation of the epidermal cells (and cells in general) in the fourth leaves. Without influencing the size of the mature cells, in -N treatment the epidermal cells in the beginning of the elongation zone were longer than in the +N plants (Fig. 2). Similar results were obtained for tall fescue and N-fertilisation (Volenec and Nelson 1983), as well as in the case of water stressed maize roots (Fraser *et al.* 1990, Sharp *et al.* 1988, Silk 1992, Sacks *et al.* 1997). The longer cells of the -N treatment were compensated by lower (estimated) number of cells in -N plants: 39 % on whole leaf basis and 18 % inside the elongation zone.

The results above suggest that the leaves of the stressed plants have shorter growth zones: the estimated length of the elongation zone was 63 mm in control plants, while in stressed plants, the length of the elongation zone was 41 mm (Fig. 3). On the other hand, the nitrogen deprivation decreased the growth velocity, implying that the cells spent more time in this shortened elongation zone (Silk 1992, Morris and Silk 1992).

The relative elemental growth rate (REGR) changes (Fig. 3*A*) indicate that the different regions of the elongation zone in a maize leaf respond variously to nitrogen deprivation. Assuming the differential effect of the nitrogen starvation on cell division and cell elongation, this might be the consequence of not altered division of cells. Analogous results were found under water stress (Fraser *et al.* 1990, Sharp *et al.* 1988, Silk 1992), salt stress (Bernstein *et al.* 1993) and even for the action of growth regulators (Chapin *et al.* 1988, Samuelson *et al.* 1992). However, it was shown that other types of stresses (for example, low temperature stress) induced a different response of REGR (Pahlavanian and Silk 1988, Silk *et al.* 1989).

During the experiments the maize seedlings were grown under relatively low irradiance (200 μmol m^{-2} s^{-1}). This equalled the P_N in +N and -N plants; consequently, the potential source of carbon originating from photosynthesis was presumably the same in +N and -N treatments. Despite the similar carbon supply, as a result of nitrogen deprivation the size and area of a single leaf decreased, as well as, the total leaf area (Tables 1, 2), which agreed with a great number of data published on the topic (MacAdam *et al.* 1989, McDonald 1989, Taylor 1993, Palmer *et al.* 1996). The total leaf area in the end of the experiment decreased by 38 %, so in order to turn comparable the source of carbon in both treatments a correction factor of 1.38 was applied to all -N values within the experiment.

The accumulation of the dry matter in +N treatment took place in the second half of the elongation zone, while close to the base the dry matter content was significantly higher in the plants supplied with sub-optimal amounts of nitrogen (Fig. 4*A*). These results correspond to the results of REGR distribution (Fig. 3), showing that inside the elongation zone the processes of cell expansion and accumulation of dry matter occur simultaneously. This slight shift towards the base of the leaf in the profile of the spatial distribution for both parameters seems to be a common feature of plant growth acclimatisation to stress conditions (Schnyder and Nelson 1988, Silk 1992). Inside the elongation zone, only close to the base of the leaf the water content of stressed plants was higher than in control plants, but afterwards the difference diminished (Fig. 4*B*), implying that nitrogen deprivation had no real effect on the water content of the tissues inside the elongation zone (Palmer *et al.* 1996). Outside the elongation zone, the water content of the leaf significantly differed in favour of the control treatment, probably due to the control plants being much thicker.

Relying on Silk's model (1984) based on the application of the continuity equation, the local net deposition rates (NDR) of dry matter and water were calculated. In control plants, the first peak in the dry matter NDR along the leaf blade was observed at 20 - 30 mm from the leaf base (Fig. 5*A*). This coincided with the start of decrease in the spatial distribution of the REGR (Fig. 3*A*). Probably this is the point where the elongation of the cell gradually stops due to the increase of cell wall rigidity (MacAdam and Nelson 1987, Schnyder and Nelson 1988, MacAdam *et al.* 1989). The second maximum can certainly be explained with the increase in the cross section of the leaf and secondary cell wall depositions (MacAdam and Nelson 1987). There is also evidence that the volumetric growth of the second peak could be attributed to dry matter deposition in light, which is probably the accumulation of assimilates from photosynthesis (Gandar 1980, Schnyder and Nelson 1987, 1988), or may coincide with secondary wall deposition, too (MacAdam and Nelson 1987).

Our experiments suggest that the leaf growth of maize seedlings, interrelated with dry matter and water accumulation, is highly affected by nitrogen deprivation and it seems as if this effect is more profound in the elongation zone. Thus the elongation zone, which is supplied more abundantly by assimilates than other parts of a growing leaf, maintains the growth of the leaf as long as possible, even under unfavourable conditions, like nitrogen deprivation. Despite the correction that made the carbon source for both treatments similar, the growth of leaves in the seedlings supplied with sub-optimal amount of nitrogen showed a decrease in the elongation and accumulation of the dry matter. Indeed, the availability of nitrogen limits growth in most environments, but the restricted development of the nitrogen-deficient plants is probably due to a lower rate of leaf expansion, rather than a decline in the rate of photosynthesis per unit leaf area.

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