

## Effects of N fertilizer on root growth in *Zea mays* L. seedlings

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### Abstract

This paper reports the effects of different nitrogen (N) fertilizer levels on the growth of maize (*Zea mays* L.) under field conditions. The N supply was found to influence the growth of the plants, especially the roots. A high N supply significantly inhibited root elongation, and was associated with reduced root dry weight compared to controls and to plants grown with smaller supplies of N. However, no differences were seen in lateral primary root density under the different N supply conditions, nor did plant N concentration increase with high N supply. In conclusion, a high N supply not only wastes resources and pollutes the environment, it may also inhibit root growth.

**Additional key words:** high nitrogen supply; inhibition of maize growth; root elongation; root initiation.

### Resumen

#### Efectos de la fertilización nitrogenada sobre el crecimiento de las raíces en plántulas de *Zea mays* L.

Se llevaron a cabo experimentos de campo para estudiar el efecto de los niveles de fertilizante de nitrógeno (N) sobre el crecimiento de maíz (*Zea mays* L.). Los resultados demostraron que el suministro de nitrógeno puede influir en el crecimiento de las plantas, especialmente en las raíces. En condiciones de campo, un elevado suministro de N inhibió significativamente la elongación de las raíces. El peso seco de las raíces que crecieron bajo un elevado suministro de N disminuyó comparado con controles o con plantas que crecieron con niveles inferiores de N. Sin embargo, la densidad de las raíces laterales primarias se mantuvo constante cuando las plantas crecieron con diversos niveles de N, y la concentración interna de N de las plantas no aumentó con un elevado suministro de N en el suelo. Un aporte elevado de N no solo desperdicia recursos y contamina el medio ambiente, sino que también puede impedir el crecimiento de las raíces.

**Palabras clave adicionales:** alargamiento de raíces, iniciación de raíces, elevado suministro de nitrógeno, inhibición de crecimiento del maíz.

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### Introduction

Unlike animals, plants cannot change their growth position, but have to adjust and adapt to environmental change *in situ*. Roots are very important in this regard: they not only absorb water and nutrients, but also release substances such as organic acids to enable optimal plant growth. The below-ground environment where plants assimilate water and nutrients is very heterogeneous, both spatially and temporally. For example, inorganic nitrogen (N) concentrations may differ 1000-fold over a distance of centimetres or over short time periods (Bloom, 1997). Therefore, plants must develop

sophisticated mechanisms that enable them to modify their morphology in response to environmental changes (Forde, 2002). This plasticity is very important to the immobile plant, allowing it survive in non-ideal conditions and successfully compete for resources (Callahan *et al.*, 1997; Malamy, 2005). Nevertheless, plasticity poses some risks. It requires some cells remain undifferentiated or that they de-differentiate to form stem cell populations, with the inherent danger of overgrowth or disorganised growth. However, this mechanism can regulate organ formation over the lifetime of the plant and help it avoid danger (Malamy, 2005).

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The mechanisms that determine root system architecture can be divided into two broad categories: (1) pathways that are essential for organogenesis and growth and that determine the characteristic architecture of the plant - the “intrinsic” pathways, and (2) pathways that determine how plants respond to external signals to modulate the “intrinsic” pathways - the “response” pathways (Malamy, 2005).

N, a necessary nutrient, is one of the most critical elements limiting plant growth because of its commonly low availability. The most common form of N in aerobic soils is nitrate, but it is highly soluble in the soil solution and can easily be lost by leaching or by bacterial denitrification (Vance, 2001). As the distribution of N is very heterogeneous, the plant must alter its root system architecture to assimilate nutrients (Lopez-Bucio *et al.*, 2003). Changes in root growth can be divided into three key stages: (1) founder cell selection/initiation, (2) development and emergence of the lateral root (LR) primordia/apical meristem activation, and (3) LR growth and orientation (Malamy, 2005). N can affect root growth either directly in the form of an external signal, or indirectly through changes in internal status (Forde and Lorenzo, 2001). Scheible *et al.* (1997) found that high levels of nitrate in shoots inhibited starch synthesis and reduced root sugar levels. Thus, root growth can be inhibited by high nitrate supply. However, as plant growth can be influenced by interactions with other environmental variables, experimental conditions can influence the observed responses (Forde and Lorenzo, 2001). In addition, phenotypic changes in plants can have impacts on their nutrient status (Colema *et al.*, 1994).

Little research has been performed on root growth inhibition due to high N supply. However, the results of work performed in solution culture (Diao and Qian, 2008) suggest the need to further investigate the effect of high N supply on root growth. In this study, the response of root morphology to N was investigated under field conditions.

## Material and methods

The N fertilizer used in this study was  $\text{CO}(\text{NH}_2)_2$  (PetroChina Ningxia Petrochemical Company, Yinchuan City, Ningxia Province, P.R. China), in which the total N content is no less than 46.4%. The P fertilizer used was superphosphate (Yunnan Honglin Chemicals Co., Ltd., Kaiyuan City, Yunnan Province, P.R. China),

which contains no less than 16%  $\text{P}_2\text{O}_5$ . The K fertilizer used was KCl (Ural Potassium Fertilizer Joint-Stock Co., Berezniki City, Perm, Russia), which contains no less than 60%  $\text{K}_2\text{O}$ . Finally, the Zn fertilizer used was  $\text{ZnSO}_4$  (Shandong Zouping Zhenzhong Chemicals Co., Ltd., Zouping City, Shandong, P.R. China), which contains no less than 95%  $\text{ZnSO}_4$ .

Experiments were performed in open fields at Shangzhuang Experimental Station, China Agricultural University, Beijing. The initial soil mineral N content was: 16 kg ha<sup>-1</sup> for the 0-30 cm layer, 26 kg ha<sup>-1</sup> for the 30-60 cm layer, and 20 kg ha<sup>-1</sup> for the 60-90 cm layer. The initial soil mineral P, K and organic matter contents were 11.17, 74.58 and 10.88 mg kg<sup>-1</sup> respectively. The pH was 8.13 (Hu *et al.*, 2006). Each treatment was replicated four times (to ensure at least three could be used). Maize (*Zea mays* L.) cv. Nongda108 was planted on 14 August 2007 at a density of 51,000 plants ha<sup>-1</sup>. Plants were subjected to one of four treatments: CK, N100, N200 or N400, i.e., 0, 100, 200, and 400 kg N ha<sup>-1</sup> respectively. N fertilizer (46.4% N) was applied on 14 August. The P (content 16%) and K (content 60%) application rates were 90 and 80 kg ha<sup>-1</sup> respectively.  $\text{ZnSO}_4$  (content 95%) was added at 15 kg ha<sup>-1</sup> to each plot. The surface area of each plot was 20 m<sup>2</sup> (4 × 5 m). Harvesting was performed on 24 August, 31 August, 7 September and 20 September. Each time, one plant was harvested from each plot.

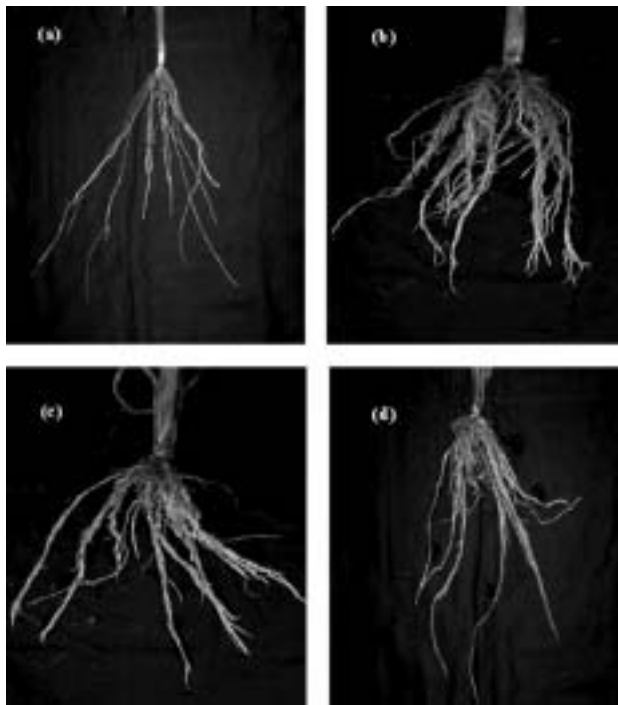
After harvest, the shoots were separated from the roots. The latter were then divided into primary, secondary and crown roots; the LRs of the primary root were counted by eye. The length of the root was scanned and measured using an Epson 4990 scanner and WinRhizo Pro Vision 5.0a software. The shoot:root length ratio was then calculated. Finally, shoots and roots were dried at 70°C for 2 d and their dry weight determined.

For measuring total N, the dried plants were digested by  $\text{H}_2\text{SO}_4\text{-H}_2\text{O}$ , and assayed using the micro-Kjeldahl protocol.

All data were analysed using SAS software.

## Results

High levels of N are known to accelerate maize growth under field conditions. However, in the present experiment, the highest N treatment had an inhibitory effect; Fig. 2 shows this effect at the third harvest. Figure 1 shows that the maize roots grew optimally in the N100 and N200 N treatments, whereas in the N400 tre-

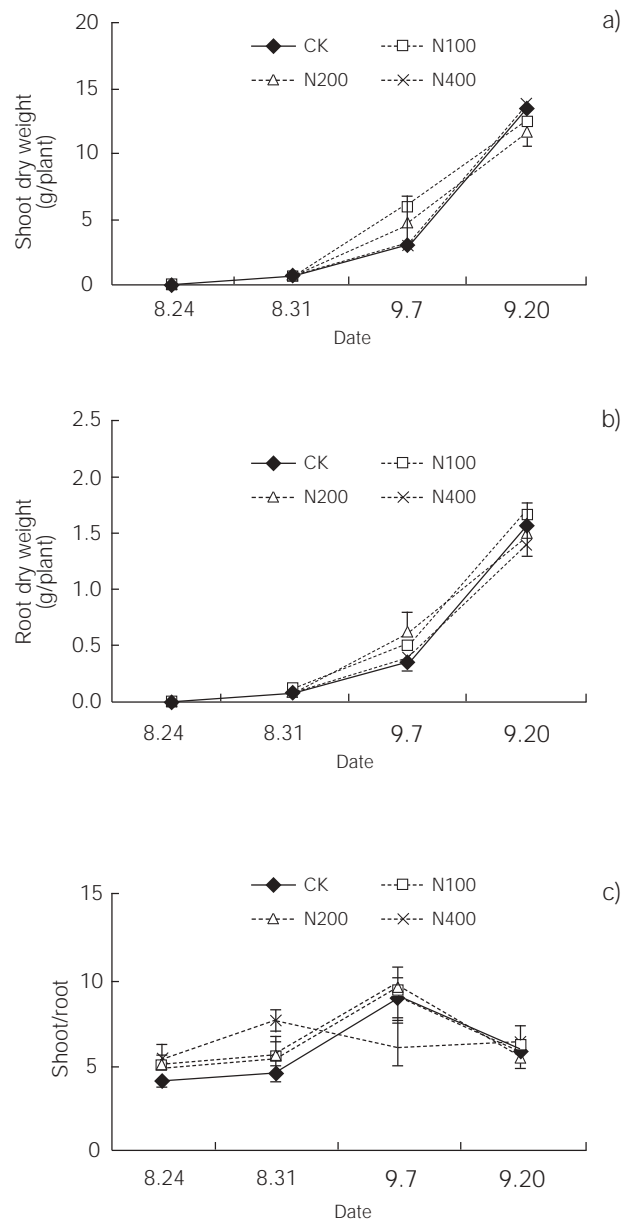


**Figure 1.** Effects of the different N treatments on maize root growth (7 September; third harvest). (a) CK, (b) N100, (c) N200, (d) N400.

atment they showed limited root growth. This inhibition was clear to the naked eye. The photographs shown (Fig. 1) are for the third harvest (7th September).

Similarly, the dry weight achieved by the plants grown under the N400 conditions was lower than in the other N treatments (Fig. 2). Figure 2a shows the mean shoot dry weight for the N400 treatment plants harvested at 31 August to be 0.46 g — significantly lower ( $P < 0.05$ ) than that recorded in the N100 treatment (0.73 g). On 7 September the shoot dry matter weighed 3.16 g, the same as in the CK treatment (3.08 g) — a value significantly different ( $P < 0.05$ ) to those recorded for the N100 and N200 treatments (5.83 g and 5.10 g respectively). High N supply also inhibited root growth. On 31 August, the mean dry weight of the roots of the N400 treatment plants was 0.06 g — significantly lower ( $P < 0.05$ ) than the results recorded for the N100 (0.17 g) and N200 treatments (0.13 g).

Figure 2c shows that the plants grown with high levels of N had a high shoot:root ratio, especially at the 24 August and 31 August harvests. On 24 August, the shoot:root ratio of the N400 plant was 5.9 — significantly higher ( $P < 0.05$ ) than in the CK treatment (4.1). For the plants harvested at 31 August, the shoot:root ratio of the



**Figure 2.** Effect of N treatments on a) shoot, b) root biomass and c) shoot:root ratio. Values represent means  $\pm$  SE ( $n = 3$  per treatment).

N400 plants was 8.1 — significantly higher ( $P < 0.01$ ) than the other three treatments (CK 4.9; N100 6.0; N200 5.9). A transient high N supply can strongly increase the shoot:root ratio. At the third harvest, many plants in the CK and N400 treatments had grown slowly and become gradually weaker; 20% had died in treatment CK, and 40% in treatment N400 by the time of the last harvest. When sampling, only living, average-sized plants were

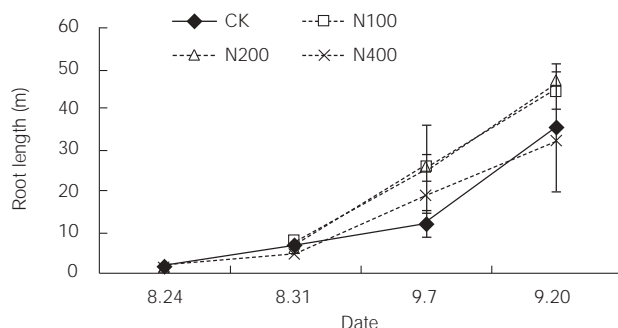
selected, which may explain why all the N treatments returned the same shoot and root dry matter results at the next sampling.

The high N supply not only influenced the plant biomass, but also root length. Figure 3 shows N400 plants had the shortest roots at all harvests except for that of 7 September. The root lengths of the N100 and N200 plants were almost the same. No significant differences ( $P>0.05$ ) were seen between N100 and N200 treatments at any of the four sampling stage. No significant differences were seen between the LR density along the primary root ( $P>0.05$ ) between the N400 and the N100 treatment plants (Fig. 4).

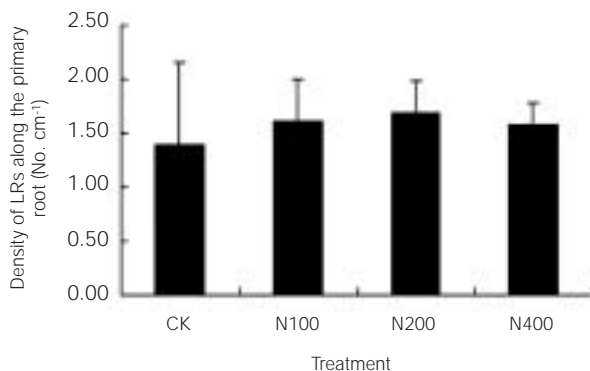
Fig. 5 shows that plant N concentrations increased after the application of N fertilizer, but no significant differences were seen among the three N treatments. This suggests that the N100 treatment is sufficient for maize growth; with greater application rates the excess N fertilizer would be released into the environment.

## Discussion

There have been extensive studies on the relationships between root growth and nutrient supply, with N and P (nutrients that have significant effects on growth) the major elements of interest. However, these studies all focused on how nutrient supply can promote plant growth. In field conditions where N supply is limited, plants can increase their root size to assimilate more soluble N from the soil (Villagarcia *et al.*, 1998). However, when the supply is sufficient it is unnecessary for plants to have large root systems, and root growth can be restricted (Robinson and Rorison, 1983; Eghball and



**Figure 3.** Effects of N supply on maize root growth. N was supplied at 0, 100, 200, or 400 kg ha<sup>-1</sup>. In all treatments, P and K were supplied at 90 and 80 kg ha<sup>-1</sup> respectively, and ZnSO<sub>4</sub> was at 15 kg ha<sup>-1</sup>. Data are means  $\pm$  SE ( $n = 3$  per treatment).

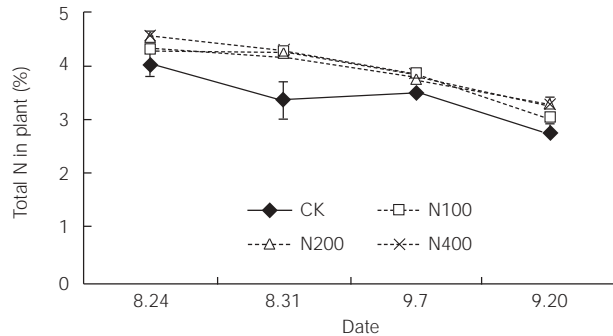


**Figure 4.** Lateral root (LR) density of the primary root at 7 September. The number of LRs was counted visually. One plant per plot was examined. Data are means  $\pm$  SE ( $n = 3$  per treatment).

Maranville, 1993). This is consistent with that seen in the present experiment, in which the total root length of the N400 plants was the smallest of all four treatments (Fig. 3). This, however, contrasts with that reported by Wang *et al.* (2004), who indicate that a high nitrate supply can increase the length of LRs, although it limits the growth of the primary root. This discrepancy can be explained in three ways. Firstly, this experiment was performed under field conditions, while Wang *et al.* worked with solutions. Secondly, the concentration of the nitrogen in the experiments of Wang *et al.* only reached 4 mM. In later work these authors found that root length was smaller when the nitrate supply was above 10 mM (Guo *et al.*, 2005). Thirdly, the nitrogen used in the present work was urea, whereas in the experiment of Wang *et al.* (2004) it was nitrate. Nitrate can induce LR elongation, acting as a signalling substance (Zhang and Forde, 1998).

Zhang and Forde (1998) suggested that the LRs of *Arabidopsis* show contrasting responses to high nitrate supply: a uniform supply of high nitrate to the whole plant may inhibit LR elongation, but a localized supply of nitrate may stimulate LR elongation within the nitrate-rich area when plants are growing in a low nitrate environment.

A uniformly high N supply under field conditions can significantly inhibit maize root growth (Zhang and Forde, 1998; Linkohr *et al.*, 2002). Linkohr *et al.* (2002) suggested that nitrate distribution stimulates LR initiation in nitrate-rich areas and suppresses root initiation in nitrate-poor areas. In the present experiment, the LR density along the primary root in the highest N supply treatment was no greater than that seen in the plants in the lower N supply treatments ( $P>0.05$ ; Fig. 4).



**Figure 5.** Effects of N supply on plant N concentration.

High nitrogen application rates also seem to inhibit shoot growth, although this was only seen at the third harvest (Fig. 2a). This might be explained by the fact that at the first and second harvests the plants were small and still using nutrients from the seed; they may therefore not have needed to absorb so many from the soil. Further, the fertilizers added to the soil had undergone leaching and volatilisation by 20 September, so the inhibition effect was not as clear as in the plants harvested at the third date.

High soil nitrogen levels seem not to commensurately increase plant N concentration (Fig. 5); this may mean plants adjust their N absorption to meet their demands. This is consistent with the research of Wang *et al.* (2004), who found no distinct correlations between the N supply and N accumulation when the N supply was high.

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