Plant Physiol. (1995) 107: 1225–1231

Regulation of Nitrate Reductase during Early Seedling Growth[1]

A Role for Asparagine and Glutamine

Sobhana Sivasankar and Ann Oaks*

Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Growth systems that either permit (wet system) or prevent (dry system) the hydrolysis of endosperm reserves in maize (*Zea mays*) seedlings were developed to study the effect of endosperm reserves on the acquisition of external nitrogen. Three-day-old seedlings treated with 5 mM KNO_3 for 24 h had higher levels of nitrate reductase (NR) activity and protein in shoot and root tissues in the dry relative to the wet system. This suggests that the induction of NR is sensitive to products of hydrolysis of endosperm reserves. Asparagine (1 mM) or glutamine (1 mM), potential products of that hydrolysis, inhibited the induction of NADH-dependent root NR in the dry system by about 70%. The inhibition of the induction of NR activity in the wet system was only about 35%, suggesting that the enzyme in the wet system was already partially repressed at 3 d. At 5 d, when asparagine and glutamine levels in the plant tissue had decreased, the induction of root NR activity was inhibited to a similar extent in the two growth systems by amide additions. The shoot enzyme was less sensitive to amide additions, and 10 mM concentrations of either amide was required for a 65% inhibition.

Amino acids and amides, derived from the hydrolysis of endosperm storage protein, satisfy the nitrogen requirements of the young seedling in the early stages of growth (Oaks and Beevers, 1964). In fact, the addition of exogenous nitrate does not contribute to the nitrogen budget of the seedling during this period when growth is supported by the endosperm (Ingle et al., 1964; Srivastava et al., 1976; Watt and Cresswell, 1987). The induction potential of NR, the first enzyme in the nitrate assimilatory pathway, is also low in the seedling at this time (Oaks, 1983; Gupta et al., 1988). Thus, there exists a constraint on the optimum expression of the nitrate assimilatory system in the seedling while it is still dependent on the organic nitrogen reserves of the endosperm. A possible reason could be that amino acids or amides, derived either directly or indirectly from the hydrolysis of storage protein, are inhibitory to nitrate assimilation.

It has been demonstrated in tissue culture systems that the development of NRA can be inhibited by the addition of casein amino acids or Gln, either of which can serve as a nitrogen source to support normal growth (Filner, 1966; Heimer and Filner, 1970; Oaks, 1974; Shiraishi et al., 1992). The transcription of the NR gene has also been shown to be reduced by Gln in whole plant systems (Callaci and Smarrelli, 1991; Deng et al., 1991; Vincentz et al., 1993; Li et al., 1995). Thus, current evidence clearly implicates amino acids, specifically Gln, as potential inhibitors of nitrate assimilation. However, in all of these previous experiments, relatively high concentrations of amino acids or amides were used to produce an inhibition of NR. Vincentz et al. (1993), for example, used 0.1 M Gln to inhibit the induction of tobacco leaf NR. True effector molecules within the cell would not be expected to be present in such high concentrations (see Oaks, 1983, for a discussion of this point).

To determine whether the amino acids released during endosperm hydrolysis might play a role in inhibiting nitrate reduction, two growth systems were developed in maize (*Zea mays*): one that permitted normal hydrolysis in the endosperm (the wet system) and the other that restricted this hydrolysis (the dry system). Growth of maize seedlings, production of NRP, and rate of NRA were compared in seedlings from each growth system.

MATERIALS AND METHODS

Growth Conditions of Plants

Maize (*Zea mays*; Pioneer hybrid No. 3475) seeds were allowed to imbibe for 5 to 6 h and then allowed to germinate in the dark on paper towels soaked in a modified one-tenth-strength Hoagland solution that contained no combined nitrogen. After 48 h, the seedlings were transferred to either a wet or dry hydroponic system containing one-tenth-strength Hoagland solution. Plastic containers with a 1.5-L capacity were used for both hydroponic systems. In the wet system, germinated seedlings were placed on a plastic mesh attached to a wooden frame, which floated on the nutrient solution. In the dry system, seedlings were placed into holes punched in the plastic lids of the growth containers. These lids retained the endosperms 0.5 cm above the solution surface, and the seedling roots

[1] This work was supported by a Canadian Commonwealth Scholarship awarded to S.S. and by a grant to A.O. from the Natural Science and Engineering Research Council of Canada (A2818).

* Corresponding author; e-mail aoaks@uoguelph.ca; fax 1–519–767–1991.

Abbreviations: NR, nitrate reductase; NRA, nitrate reductase activity; NRP, nitrate reductase protein.

passed through the holes into the nutrient solution below. In both systems the nutrient solution was aerated.

Seedlings were grown in growth chambers under a 16-h/8-h day/night regime, at day/night temperatures of 28/26°C, and under a light intensity of 225 μE m^{-2} s^{-1}. They were induced with 5 mM KNO_3 20 or 24 h before harvest and harvesting was always done 4 to 5 h into the light period. In the experiments in which amide additions were made, Asn (1 mM) or Gln (1 mM) was added to the hydroponic system 2 h before the nitrate additions. In these experiments control plants received 5 mM KNO_3 but no amide. Also, streptomycin sulfate (250 μg mL^{-1}), penicillin (K^+ salt; 10 μg mL^{-1}), and chloramphenicol (10 μg mL^{-1}) were added to the growth medium in these experiments. Shoots above the second node and roots below the first node were harvested, frozen in liquid nitrogen, ground to a fine powder, and stored at −70°C. Tissues were used for NRA and NRP assays within 3 d of harvest.

Assay for NRA

Tissue samples were extracted at 4°C using an extraction buffer containing 25 mM Tris-HCl (pH 8.5), 1 mM EDTA, 20 μM FAD, 1 mM DTT, 1% (w/v) BSA, and 10 mM Cys. The protease inhibitors chymostatin (10 μM, dissolved in DMSO) and leupeptin (10 μM) were added to the extraction buffers for root and shoot tissue, respectively. One gram of frozen tissue was ground with 4 mL of extraction buffer in a mortar. The extracts were then centrifuged at 10,000 rpm for 25 min, filtered through Miracloth, and assayed for enzyme activity. NRA was assayed as previously described by Long and Oaks (1990) and is expressed as μmol nitrite produced h^{-1} g^{-1} fresh weight. In maize roots, which possess an NADH-NR- as well as an NAD(P)H-bispecific NR, the estimate of the bispecific enzyme activity was measured with NADPH. Values indicating NADH-NRA in roots represent the combined activity of both enzymes.

Western Blot Analysis of NRP

Shoot and root tissues were extracted for western blot analysis using a buffer that was identical with the extraction buffer described above, except that it did not contain BSA. The proteins in the extract were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with a polyclonal antibody to NR as described by Li and Oaks (1993). This antibody recognizes both the NADH- and the NAD(P)H-bispecific forms of the enzyme (Long and Oaks, 1990). The reaction with the antibody is expressed only in crude extracts prepared from plants grown in the presence of nitrate and is lost when a relatively pure preparation of NRP was allowed to compete with the antibody (Fedorova et al., 1994).

Endosperm Nitrogen and Carbon Contents

Endosperms without the scutella were dried to a constant weight and ground to a fine powder before analysis. The content of nitrogen and carbon were determined using a Carlo Erba (Milan, Italy) Nitrogen Analyser (model NA 1500), which measured elemental nitrogen and carbon dioxide released by the complete combustion of the sample. A thermal conductivity detector was used and the instrument was calibrated with atropine as a standard.

Tissue Amide Analysis

Shoot and root tissues were extracted with 80% ethanol and the Asn and Gln in the extract were separated and quantitated using a Beckman HPLC (model 421) and a Gilson (Middleton, WI) fluorometer (excitation at 360 nm and emission at 455 nm). A Beckman ultrasphere ODS reverse-phase column with 5-μm particles and dimensions of 4.6 mm × 25 cm was used. The amino acids derivatized with *o*-phthalaldehyde were separated according to the method described by Winspear and Oaks (1983).

RESULTS

Effect of Growth System on the Hydrolysis of Endosperm Reserves

Maize seedlings were grown in either a wet or a dry hydroponic system for 8 d. Endosperm hydrolysis was effectively prevented in the dry growth system as shown by the stable endosperm dry weight and total nitrogen and total carbon contents during the 8-d growing period (Fig. 1). In the wet system, the endosperm dry weight and nitrogen and carbon contents decreased rapidly. Nitrogen reserves, for example, were depleted to 48% of the initial levels by 3 d in the wet system, but in the dry system they were not significantly different from the initial values.

Effect of Growth System on Embryo Growth

In both the wet and dry systems, there was a gain in total plant dry weight during the 8-d growth period. The shoot system in either case was composed of three leaves, and the root system was well developed. The shoot and root dry weights of seedlings grown in the wet and dry systems were not appreciably different 3 d after imbibition, but at later times both parameters were higher in seedlings grown in the wet system (Fig. 2). The seedlings in this system also produced more lateral roots (data not shown). In the wet system, the loss in endosperm dry weight during the 8-d period was of a magnitude similar to the gain observed in seedling dry weight.

NRA as Influenced by the Hydrolysis of Endosperm Reserves

The activity of NR in the wet and dry growth systems followed a similar trend with age. NRA was not detected in seedlings growing in a minus-nitrate medium (Table I; Fedorova et al., 1994). Seedlings induced with 5 mM KNO_3 for 24 h had low levels of NRA in both shoot and root tissues on the 3rd and 4th d after imbibition. The maximum activities of shoot NR, root NADH-NR, and root

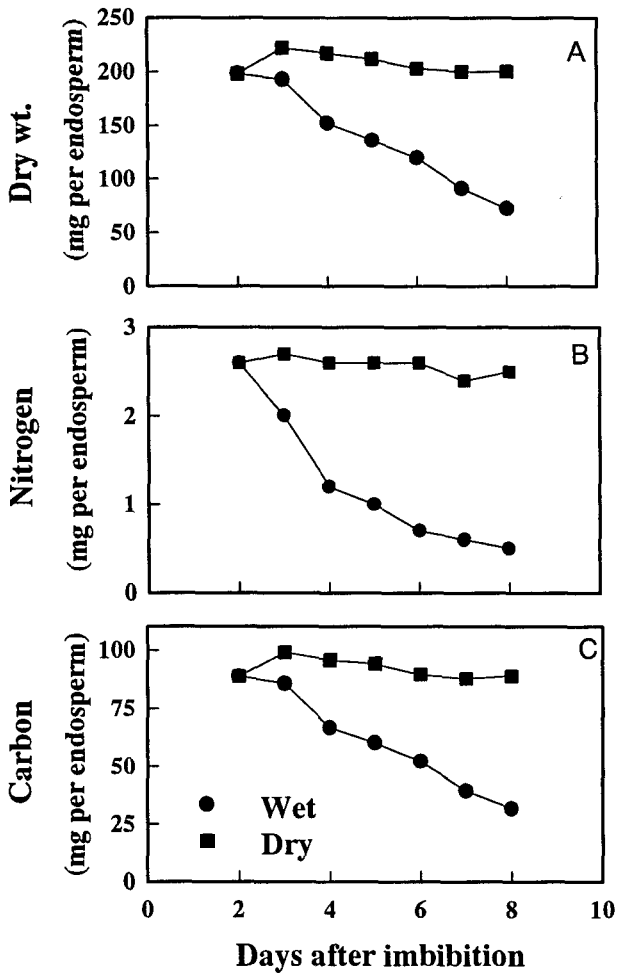

Figure 1. The effect of the wet (●) and dry (■) growth systems on the dry weight (A), nitrogen (B), and carbon (C) content of the endosperm of corn seedlings during the period from 2 to 8 d after imbibition. Values are the means of two separate experiments.

NADPH-NR were reached by the 5th or 6th d, after which time there was a decline (Fig. 3).

The shoot and root NR activities were always higher (sometimes more than 2-fold) in 3-d-old seedlings grown under dry relative to wet conditions (Table I). This coincided with the period of rapid depletion of endosperm nitrogen reserves in the wet system. After the 3rd d, levels of both shoot and root NRA increased at a faster rate in the wet system relative to the dry system, and by d 5 they were higher in the wet system. The levels of NRP in shoots and roots of 3-d-old seedlings were also higher in the dry system than in the wet system (Fig. 4, lanes 1 and 2). The difference in activity between 3-d-old wet and dry seedlings depended on the extent of immersion of the endosperms in the hydroponic solution in the wet system and the period for which the seeds were allowed to imbibe before being placed for germination. When the system was standardized and endosperm completely submerged, there was always a clear difference between the wet and dry systems in the level of NR activity and protein at d 3, as indicated in Table I and Figure 4 (lanes 1 and 2).

Effect of Amides on NRA and NRP

The addition of 1 mM Asn or 1 mM Gln to the growth medium 2 h before nitrate addition caused an inhibition in the induction of root NRA. In 3-d-old seedlings, root NADH-NRA was inhibited by about 70% in the dry system, whereas the inhibition in the wet system was only about 35% (Fig. 5A). A similar effect was apparent with the root NADPH-NR as well. Additions of Asn or Gln to shoot and root extracts of control plants had no effect on NR activity (data not shown). By the 5th d, the inhibition of root NADH-NR in the wet system increased to 68%, whereas that in the dry system remained at about 83% (Fig. 5B). The inhibition of shoot NR was less in both systems and did not change with age. This observation suggests (a) that Asn and Gln are not as efficiently transported to the shoot from the root as from the endosperm or (b) that some factor in the endosperm other than Asn or Gln is contributing to the repression of the shoot NR. The induction of NRP was also inhibited by the addition of the amides (Fig. 4, lanes 3–5). The development of root NRP was almost completely inhibited by both Asn and Gln. The shoot NRP was also repressed by amide additions, but the effect was less severe than in the roots.

Amide concentrations as low as 0.01 mM were sufficient to cause an inhibition of root NRA in 5-d-old seedlings grown in the dry system and 1 mM caused a maximum inhibition. The inhibition of NRA in shoot tissues increased over a range of 0.1 to 10 mM (Fig. 6). The inhibition increased to about 65% in the shoot when the concentration of either amide in the external medium was 10 mM. Amide concentrations of 0.1 M, which inhibited NR in excised tobacco shoots (Vincentz et al., 1993), resulted in wilting in

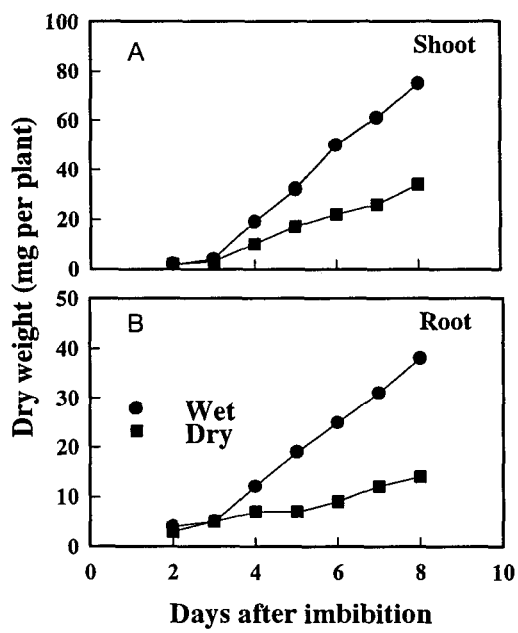

Figure 2. The effect of the wet (●) and dry (■) growth systems on the dry weight of the shoot (A) and root (B) of corn seedlings during the period from 2 to 8 d after imbibition. Values are the means of two separate experiments.

Table I. *The effect of the wet and dry growth systems on the activities of shoot NADH-NR, root NADH-NR, and root NADPH-NR of corn seedlings at 3 and 5 d after imbibition*

Shoots and roots of plants that received no nitrate addition had NR activities of <0.5 and <0.1 μmol nitrite g^{-1} fresh weight h^{-1}, respectively. The values represent means ± SE of five separate experiments and statistical significance (*) was assessed using Student's *t* test ($P < 0.05$, with 8 degrees of freedom). NS, Nonsignificant.

Treatment	Shoot NADH-NRA	Root NADH-NRA	Root NADPH-NRA
		μmol nitrite g^{-1} fresh wt h^{-1}	
3 d old, wet	1.3 ± 0.2	1.6 ± 0.3	0.9 ± 0.4
3 d old, dry	3.9 ± 0.9	3.6 ± 0.7	2.5 ± 0.8
Significance	*	*	*
5 d old, wet	6.3 ± 0.7	6.1 ± 1.3	3.0 ± 1.2
5 d old, dry	4.4 ± 0.6	3.9 ± 1.3	2.4 ± 1.2
Significance	*	NS	NS

the case of excised maize shoots and only minor inhibition of NRA and NRP (Li et al., 1995).

Tissue Amide Contents

In seedlings grown in the wet system without any external amide additions, the content of Asn and Gln in shoot and root tissues were more than 2-fold higher at 3 d than at 5 d (Table II). Asn levels were also higher than Gln levels in both shoot and root at this stage. The amides could be derived from the hydrolysis of endosperm proteins or could be synthesized in the seedling. In either case, they are present in concentrations that are high enough to inhibit the development of both NR activity and protein in 3-d-old seedlings growing in the wet system. Addition of Asn or Gln to the growth medium led to elevated levels of the respective amide in both roots and shoots of 5-d-old seedlings growing in the dry system (Table III).

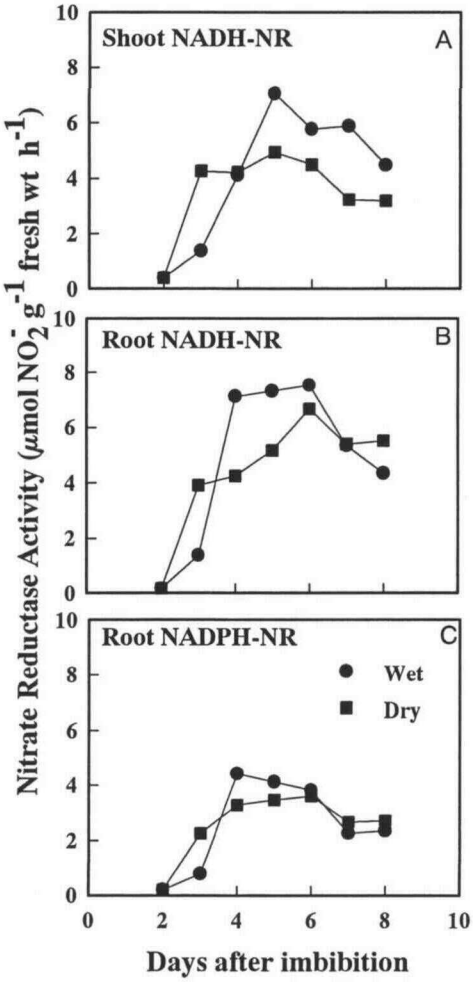

Figure 3. The effect of the wet (●) and dry (■) growth systems on the activities of shoot NADH-NR (A), root NADH-NR (B), and root NADPH-NR (C) of corn seedlings during the period from 2 to 8 d after imbibition. Values are the means of two separate experiments.

DISCUSSION

NR, the first enzyme in the nitrate assimilatory pathway, reduces absorbed nitrate to nitrite within the plant using either NADH (EC 1.6.6.1) or NADPH (EC 1.6.6.2) as the reductant. This substrate-inducible enzyme is regulated at the levels of transcription, translation, and enzyme activity by several factors including light, nitrogen source, age, and metabolites (Srivastava, 1980; Rajasekhar and Oelmuller, 1987; Hoff et al., 1992; Vincentz et al., 1993; Oaks, 1994). In maize, the activity of NR in the seedling reaches maximum levels by the 5th or 6th d after planting (Long and Oaks, 1990). The period from 0 to 4 d when the NR activity is low coincides with the period of rapid hydrolysis of endosperm nitrogen reserves (Fig. 1; Ingle et al., 1964; Harvey and Oaks, 1974). Asn and Gln, potential products of endosperm hydrolysis in maize, are predominant in the plant tissue

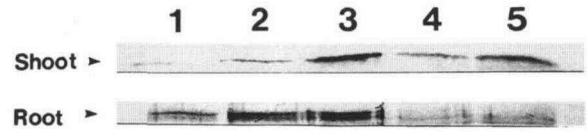

Figure 4. The effect of the wet and dry growth systems and amide additions on NRP in maize seedlings. Western blot analysis was done with tissue extracts from three separate experiments and representative results are presented. Lanes numbered serially from 1 to 5 are: 1, 3-d-old tissue grown in the wet system induced with 5 mM KNO_3; 2, 3-d-old tissue grown in the dry system induced with 5 mM KNO_3; 3, 5-d-old tissue grown in the dry system induced with 5 mM KNO_3; 4, 5-d-old tissue grown in the dry system treated with 1 mM Asn before induction with 5 mM KNO_3; and 5, 5-d-old tissue grown in the dry system treated with 1 mM Gln before induction with 5 mM KNO_3.

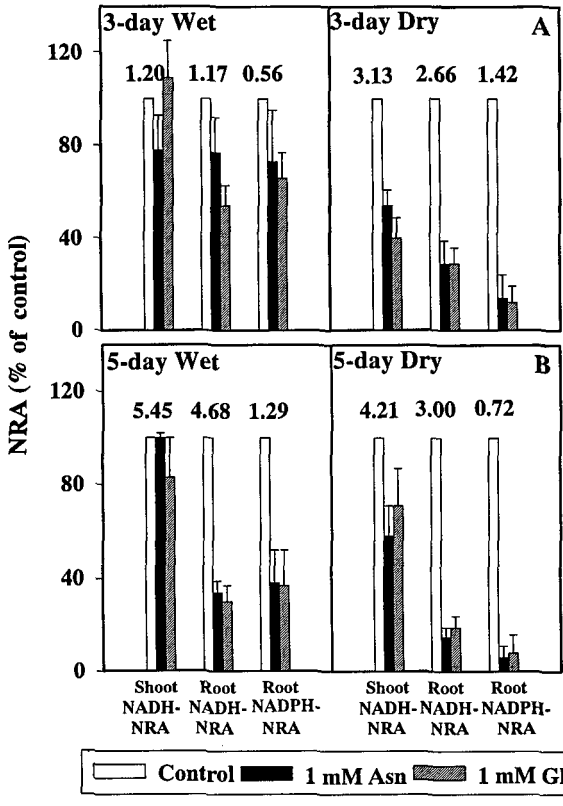

Figure 5. The effect of Asn or Gln on nitrate induction of NRA in corn seedlings (A, 3 d old; B, 5 d old) grown under the wet or dry growth systems. The actual NRA values (μmol nitrite produced h^{-1} g^{-1} fresh weight) for the control seedlings, which were set to 100%, are presented above each control bar. Results are the means ± SE of four separate experiments, and enzyme assays were performed in duplicate for each extract.

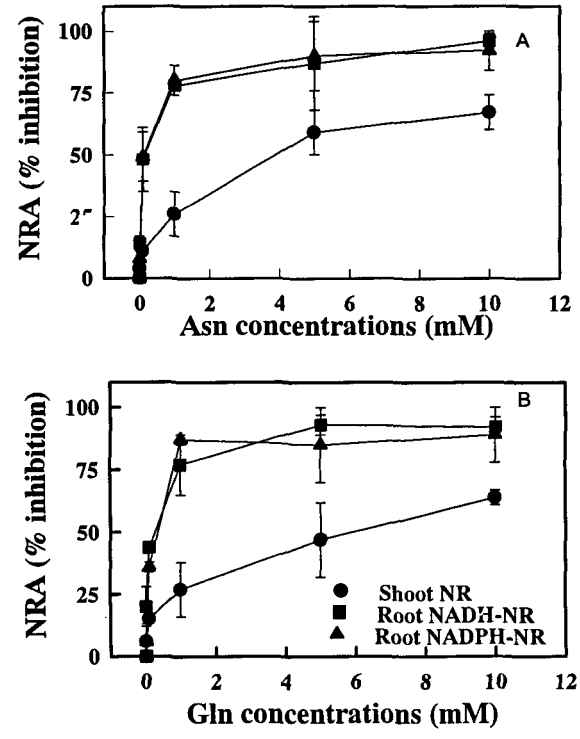

Figure 6. The effect of different concentrations of Asn (A) and Gln (B) on the inhibition of NRA in corn seedlings. Asn and Gln at 0.01, 0.1, 1.0, 5.0, or 10.0 mM were applied to 4-d-old seedlings growing in the dry system. Nitrate (5 mM KNO_3) was added 2 h later and seedlings were harvested after an induction period of 20 h. Shoot and root NRAs were assayed and the percentage of inhibition as compared to the control was determined. Values are means ± SE of three separate experiments.

and in root exudates during early seedling growth (Table II; S. Sivasankar and A. Oaks, unpublished observations). Of the two amides, Gln is known to be the effector molecule responsible for nitrogen catabolite repression of the transcription of NR in the filamentous fungus *Neurospora crassa* (Premakumar et al., 1979, 1980; Marzluf and Fu, 1989). This amide, together with Asn, constitutes more than 50% of the reduced nitrogen in the root exudate of 7-d-old maize seedlings (S. Sivasankar and A. Oaks, unpublished results). Taken together, the above facts suggest that the observed constraint on NR activity during early seedling growth resulted from an inhibition by Asn and/or Gln derived either from the hydrolysis of protein reserves in the endosperm or from their synthesis within the seedling itself.

In our experiments, endosperm hydrolysis was reduced to prevent the supply of reserve nitrogen to the growing embryo. When the hydrolysis of endosperm reserves was restricted (dry system), there was an increase in NR activity that was greater by 2-fold than that in seedlings with a normal hydrolysis of endosperm reserves (Fig. 3). The increase in NRA achieved by the removal of endosperm supply to the seedling occurred within the first 4 d, a time when the rate of depletion of endosperm nitrogen was at its highest.

Exogenous application of either Asn or Gln, at concentrations as low as 1 mM, significantly inhibited NR in both 3- and 5-d-old seedlings in the dry system. In the wet system, on the other hand, the inhibition was minor in 3-d-old seedlings (35%) but reached significant levels (68%) by 5 d. In vivo levels of Asn and Gln in these seedlings were high at d 3 but had declined to levels below 50% by d 5 (Table II). Thus, it is possible that the pools of Asn and Gln generated within the seedling in the wet system were high enough to inhibit the development of NR in the 3-d-

Table II. *The effect of the wet and dry growth systems on the Asn and Gln contents in shoots and roots of 3- and 5-d-old corn seedlings*

The experiment was repeated twice and similar trends were obtained. The data from one representative experiment are presented.

Treatment	Shoot		Root	
	Asn	Gln	Asn	Gln
	μmol g^{-1} fresh wt			
3 d old, wet	23.8	15.0	5.0	2.6
3 d old, dry	9.1	2.6	2.5	1.3
5 d old, wet	6.7	1.9	1.6	1.4
5 d-old, dry	7.5	0.8	2.0	0.3

Table III. *Amide contents in shoots and roots of 5-d-old corn seedlings grown in the dry system*

Seedlings were induced with 5 mM KNO_3 in the absence (control) or presence of 1 mM Asn or 1 mM Gln. Values represent means ± SE of three separate experiments.

Treatment	Shoot		Root	
	Asn	Gln	Asn	Gln
	μmol g^{-1} fresh wt			
Control (no amide)	3.0 ± 1.3	0.7 ± 0.1	1.8 ± 0.5	0.7 ± 0.1
1 mM Asn	6.0 ± 0.4	1.1 ± 0.1	3.1 ± 1.1	1.6 ± 0.7
1 mM Gln	5.6 ± 0.3	1.0 ± 0.2	3.3 ± 1.0	1.5 ± 0.3

old seedling and that because of this inhibition the roots of these seedlings were less sensitive to exogenous Asn or Gln.

In our system, root tissues were more sensitive to the inhibitory effect of amides than shoot tissues (Fig. 4). Since the amide concentrations were elevated in the shoot tissue when the seedlings were exposed to exogenous amides, there was an efficient transfer from root to shoot (Table III). Therefore, the lower levels of inhibition of NR in the shoot may have been due to either a reduced sensitivity of the maize shoot enzyme to the inhibitors or differences in compartmentation in shoot and root tissues. When external concentrations of either amide were increased to 10 mM the inhibition of shoot NRA (65%) was similar to that seen in root tissues with 1 mM (Fig. 6). Vincentz et al. (1993) have also shown that the expression of NR transcripts in detached tobacco leaves is completely inhibited by Gln concentrations of 0.1 M. In their system roots were more sensitive than shoots to the addition of Gln (Deng et al., 1991). In maize, the shoot NR is NADH dependent, whereas the roots possess an NADH-NR as well as an NAD(P)H-bispecific NR (Long et al., 1992). Thus, it is possible that the NAD(P)H-bispecific NR, which appears to be the predominant isozyme in maize roots, is more sensitive to inhibition by amides than the NADH-NR, leading to the differential response to amides in shoots and roots. The amide concentration used in our experiments was 1 mM, a concentration that approaches the true physiological concentration. This is much lower than those used in earlier experiments (Oaks, 1974; Shiraishi et al., 1992; Vincentz et al., 1993; Li et al., 1995). The high sensitivity of the root NR seen in our experiments could be due to the age of the plant as well as the manipulation of plant growth. Limiting the endosperm supply of nitrogen should and does result in lower pool sizes of mobilized nitrogen metabolites in the growing seedling. This, in turn, should increase the capacity of the plant for assimilation of external nitrate if internal nitrogen is repressing mechanisms of uptake and assimilation of external nitrate. External application of amides to these plants should, and in the case of maize roots does, impose an inhibition in situ similar to the one seen under normal seedling growth, the situation found in our wet system.

It is evident from the results presented here that the low capacity for assimilation of external nitrate by growing tissues such as seedlings (or meristems or metabolic sinks) could result, in part, from an inhibition of the induction of NR by nitrogenous compounds that are actively imported from senescing plant parts. We think that these transported metabolites are essential for permitting rapid growth in such tissues by facilitating the synthesis of those proteins required to establish the structural and functional integrity of the new cell. A repression of NR in these tissues would prevent an unnecessary drain of reductant, energy, and carbon into the nitrate assimilation pathway, resources that would otherwise be utilized for new growth.

Received August 5, 1994; accepted December 15, 1994.
Copyright Clearance Center: 0032-0889/95/107/1225/07.

LITERATURE CITED

Callaci JJ, Smarrelli J Jr (1991) Regulation of the inducible nitrate reductase isoform from soybeans. Biochim Biophys Acta **1088:** 127–130

Deng M, Moureaux T, Cherel I, Boutin J-P, Caboche M (1991) Effects of nitrogen metabolites on the regulation and circadian expression of tobacco nitrate reductase. Plant Physiol Biochem **29:** 239–247

Fedorova E, Greenwood JS, Oaks A (1994) *In-situ* localization of nitrate reductase in maize roots. Planta **194:** 279–286

Filner P (1966) Regulation of nitrate reductase in cultured tobacco cells. Biochim Biophys Acta **118:** 299–310

Gupta AK, Sharma AK, Guha-Mukherjee S, Sopory SK (1988) Inhibition of nitrate reductase in germinating barley embryos by endosperm. Plant Sci **54:** 141–146

Harvey BMR, Oaks A (1974) The hydrolysis of endosperm protein in *Zea mays*. Plant Physiol **53:** 453–457

Heimer YM, Filner P (1970) Regulation of the nitrate assimilation pathway of cultured tobacco cells. II. Properties of a variant cell line. Biochim Biophys Acta **215:** 152–165

Hoff T, Stummann BM, Henningsen KW (1992) Structure, function and regulation of nitrate reductase in higher plants. Physiol Plant **84:** 616–624

Ingle J, Beevers L, Hageman RH (1964) Metabolic changes associated with the germination of corn. I. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum and endosperm. Plant Physiol **39:** 735–740

Li X-Z, Larson DE, Glibetik M, Oaks A (1995) Effects of nitrogen metabolites on the induction of maize nitrate reductase. Physiol Plant (in press)

Li X-Z, Oaks A (1993) Induction and turnover of maize nitrate reductase. Influence of NO_3^-. Plant Physiol **102:** 1251–1257

Long DM, Oaks A (1990) Stabilization of nitrate reductase in maize roots by chymostatin. Plant Physiol **93:** 846–850

Long DM, Oaks A, Rothstein SJ (1992) Regulation of maize root nitrate reductase mRNA levels. Physiol Plant **85:** 561–566

Marzluf G, Fu Y-H (1989) Genetics, regulation and molecular studies of nitrate assimilation in *Neurospora crassa*. *In* JL Wray, JR Kinghorn, eds, Molecular and Genetic Aspects of Nitrate Assimilation. Oxford Science Publications, New York, pp 314–327

Oaks A (1974) The regulation of nitrate reductase in suspension cultures of soybean cells. Biochim Biophys Acta **372:** 122–126

Oaks A (1983) Regulation of nitrogen metabolism during early seedling growth. *In* C Nozzolillo, PJ Lea, FA Loewus, eds, Mobilisation of Reserves in Germination. Plenum, New York, pp 53–75

Oaks A (1994) Primary nitrogen assimilation in higher plants and its regulation. Can J Bot **72:** 739–750

Oaks A, Beevers H (1964) The requirement for organic nitrogen in *Zea mays* embryos. Plant Physiol **59:** 37–43

Premakumar R, Sorger GJ, Gooden D (1979) Nitrogen metabolite repression of nitrate reductase in *Neurospora crassa*. J Bacteriol **137:** 1119–1126

Premakumar R, Sorger GJ, Gooden D (1980) Repression of nitrate reductase in *Neurospora* studied by using *L*-methionine-*DL*-sul-

foximine and glutamine auxotroph *gln-lb*. J Bacteriol **143:** 411–415

Rajasekhar VK, Oelmuller R (1987) Regulation of induction of nitrate reductase and nitrite reductase in higher plants. Physiol Plant **71:** 517–521

Shiraishi N, Sato T, Ogura N, Nakagawa H (1992) Control by glutamine of the synthesis of nitrate reductase in cultured spinach cells. Plant Cell Physiol **33:** 727–731

Srivastava HS (1980) Regulation of nitrate reductase activity in higher plants. Phytochemistry **19:** 725–733

Srivastava HS, Oaks A, Bakyta IL (1976) The effect of nitrate on early seedling growth in *Zea mays*. Can J Bot **54:** 923–929

Vincentz M, Moureaux T, Leydecker M-T, Vaucheret H, Caboche M (1993) Regulation of nitrate and nitrite reductase expression in *Nicotiana plumbaginifolia* leaves by nitrogen and carbon metabolites. Plant J **3:** 315–324

Watt MP, Cresswell CF (1987) A comparison between the utilisation of storage protein and exogenous nitrate during seedling establishment in *Zea mays* L. Plant Cell Environ **10:** 327–332

Winspear MJ, Oaks A (1983) Automated pre-column amino-acid analyses by reversed-phase high-performance liquid chromatography. J Chromatogr **270:** 378–382