

Role of cytokinin in enhanced productivity of maize supplied with NH_4^+ and NO_3^-

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Abstract

Supplying both N forms ($\text{NH}_4^+ + \text{NO}_3^-$) to the maize (*Zea mays* L.) plant can optimize productivity by enhancing reproductive development. However, the physiological factors responsible for this enhancement have not been elucidated, and may include the supply of cytokinin, a growth-regulating substance. Therefore, field and gravel hydroponic studies were conducted to examine the effect of N form ($\text{NH}_4^+ + \text{NO}_3^-$ versus predominantly NO_3^-) and exogenous cytokinin treatment (six foliar applications of 22 μM 6-benzylaminopurine (BAP) during vegetative growth versus untreated) on productivity and yield of maize. For untreated plants, $\text{NH}_4^+ + \text{NO}_3^-$ nutrition increased grain yield by 11% and whole shoot N content by 6% compared with predominantly NO_3^- . Cytokinin application to NO_3^- -grown field plants increased grain yield to that of $\text{NH}_4^+ + \text{NO}_3^-$ -grown plants, which was the result of enhanced dry matter partitioning to the grain and decreased kernel abortion. Likewise, hydroponically grown maize supplied with $\text{NH}_4^+ + \text{NO}_3^-$ doubled anthesis earshoot weight, and enhanced the partitioning of dry matter to the shoot. $\text{NH}_4^+ + \text{NO}_3^-$ nutrition also increased earshoot N content by 200%, and whole shoot N accumulation by 25%. During vegetative growth, $\text{NH}_4^+ + \text{NO}_3^-$ plants had higher concentrations of endogenous cytokinins zeatin and zeatin riboside in root tips than NO_3^- -grown plants. Based on these data, we suggest that the enhanced earshoot and grain production of plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$ may be partly associated with an increased endogenous cytokinin supply.

Introduction

Hydroponic and growth-chamber studies have demonstrated that $\text{NH}_4^+ + \text{NO}_3^-$ nutrition can often increase growth or grain yield of maize (Antil et al., 1983; Below and Gentry, 1987; Schrader et al., 1972). Although the physiological processes that are altered by $\text{NH}_4^+ + \text{NO}_3^-$ nutrition have yet to be fully elucidated, factors associated with reproductive development have been implicated. For example, Below and Gentry (1987) noted that plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$ partitioned a larger amount of dry matter to the grain. In addition, Camberato and

Bock (1989) found that sorghum (*Sorghum bicolor* (L.) Moench.) plants grown with both N forms increased floret and kernel numbers compared with NO_3^- nutrition. Because endogenous cytokinins have been implicated in the regulation of tiller formation and survival in cereals (Michael and Beringer, 1980), and in the establishment of kernel sink capacity (Wheeler, 1972), cytokinin supply is one factor that could be related to enhanced reproductive development induced by $\text{NH}_4^+ + \text{NO}_3^-$ nutrition.

Previous work has shown that N supply can alter cytokinin synthesis and production by the plant. A restricted mineral supply to *Plantago*

major ssp. *pleiosperma* plants was associated with decreased cytokinin production, but this deficiency could be partially alleviated by exogenous benzyladenine application (Kuiper et al., 1989). Similarly, cytokinin application to one side of wheat (*Triticum aestivum* L.) plants grown in a 'split' root system resulted in increased N retention by the shoot under NO_3^- -N limiting conditions (Simpson et al., 1982). When examining different N-form regimes, Salama and Wareing (1979) reported that NO_3^- -N stimulated plant growth and increased the endogenous zeatin concentration in exudate from sunflower (*Helianthus annuus* L.) roots, compared with NH_4^+ -N nutrition. In contrast, when NH_4^+ -N fertilization did not inhibit the growth of young apple (*Pyrus malus*) rootstocks, higher zeatin concentrations were found in xylem exudate compared with a NO_3^- -N supply (Buban et al., 1978). Thus it appears that N fertility regimes that promote plant development are also associated with higher quantities of cytokinin.

Despite the known importance of cytokinin in plant growth and development, few studies have critically investigated the physiological impact of exogenous cytokinin application. For example, activities of nitrate reductase (Roa et al., 1984) and glutamine synthetase (Ghisi and Passera, 1987), and the spikelet number per tassel (Polowick and Greyson, 1984) were stimulated by exogenous cytokinin. In addition, exogenous cytokinin application increased chlorophyll and protein concentrations of big bluestem (*Andropogon gerardi* Muhl.) (Towne and Owensby, 1983) or leaf oil content of several species of the *Lamiaceae* family (El-Keltawi and Croteau, 1987). Likewise, pea (*Pisum sativum* L.) seedlings treated with benzyladenine were more frost-tolerant than water-sprayed controls (Kuraishi et al., 1966). However, as might be expected with exogenous plant growth regulator applications, Schistad and Nissen (1984) noted that the application dosage could alter the response of senescing barley (*Hordeum sativum* L.) to cytokinin. For maize, Koter et al. (1983) reported that kinetin applied to the leaves prolonged vegetative growth, which resulted in decreased grain yield. Collectively, these studies indicate that the application of exogenous cyto-

kinin can alter many processes that encompass growth and physiology.

Although the physiological processes altered by $\text{NH}_4^+ + \text{NO}_3^-$ nutrition have not been fully elucidated, evidence indicates that $\text{NH}_4^+ + \text{NO}_3^-$ results in increased kernel number and greater partitioning of assimilate to the grain. An increased supply of cytokinin may be partially responsible for the enhanced reproductive development, because cytokinin plays a major role in reproductive tissue formation. Therefore, field and gravel hydroponic studies were conducted to elucidate plant processes altered by $\text{NH}_4^+ + \text{NO}_3^-$ nutrition, and to investigate the role of cytokinin when both N forms were supplied to the maize plant compared with NO_3^- alone. We hypothesized that $\text{NH}_4^+ + \text{NO}_3^-$ nutrition would stimulate the synthesis and supply of cytokinin, thus increasing reproductive potential and grain yield.

Materials and methods

Field study

The maize genotype B73 \times LH51 was evaluated in 1989 and 1990 to determine if cytokinin applied to the leaves could alter growth or grain productivity of plants supplied with either $\text{NH}_4^+ + \text{NO}_3^-$ or NO_3^- nutrition. Treatments were arranged in a randomized complete block factorial design, with two replicates in 1988 and four replicates in 1989. The hybrid B73 \times LH51 was evaluated because of its consistent yield increase when supplied with both forms of N compared with NO_3^- alone (Below and Gentry, 1987). Kernels of B73 \times LH51 were planted on 13 May 1989, or on 27 April 1990 and thinned 14 days after emergence to 63 000 plants ha^{-1} . Each plot consisted of four 2.2 m rows spaced 76 cm apart. Plots were located at the Agronomy Cruse Research Farm (Champaign, IL, USA), on soil classified as Drummer silty clay loam (fine, silty, mixed, mesic, Typic Haplaquoll; soil pH 5.6 and 3.0% organic matter). An overhead irrigation system supplied water as needed throughout the growing season.

At the third leaf stage (V3; Ritchie et al., 1986), 224 kg N ha^{-1} was applied in granular form, either as calcium nitrate (NO_3^- plots) or

urea ($\text{NH}_4^+ + \text{NO}_3^-$ plots). For the $\text{NH}_4^+ + \text{NO}_3^-$ plots, a large proportion of the N was maintained as NH_4^+ form by the application of an experimental nitrification inhibitor along with the urea. The inhibitor was not applied to NO_3^- plots in order to minimize the possibility of ammonification not being followed by nitrification. In both years, N fertilizer treatments were incorporated into the soil, via immediate cultivation and the application of 2.5 cm of irrigation water. At various intervals during the growing season, soil from each plot (which contained plants) was sampled, and a 2M KCl soil extract analyzed for NO_3^- (Norman et al., 1985) and NH_4^+ (Cataldo et al., 1974). At the V4, V6, V8, V10, V14, and V18 plant developmental stages, approximately 1 L of 22 μM 6-benzylamino-purine (BAP) plus 0.01% Tween 80 surfactant were applied to the entire vegetative portion (leaves and stalk) of all plants within the two interior rows of the treated plots. The plots which did not receive BAP treatment were treated with a similar amount of a solution containing only 0.01% Tween 80. To minimize ultraviolet degradation of the cytokinin, all applications were made at twilight.

At the seven-leaf stage (V7), anthesis (R1), and physiological maturity (R6), four plants were harvested from each plot to determine dry weight and nutrient content (Heberer et al., 1985; Swank et al., 1982). The number of ovules at anthesis was determined from the harvested R1 earshoots (which exhibited visible silks) by counting the number of ovule rows and multiplying by the number of ovules (pollinated and unpollinated) in a representative row. The magnitude of kernel abortion was determined by dividing R6 kernel number by the number of ovules at anthesis.

Field and laboratory data were analyzed utilizing a combined-year factorial analysis of variance (ANOVA), with years and replicates considered random and treatments fixed. Treatment means were compared using Fisher's least significant difference (LSD) at the 0.05 probability level.

Hydroponic study

Gravel hydroponic techniques were used to evaluate the effect of $\text{NH}_4^+ + \text{NO}_3^-$ nutrition on

vegetative and early reproductive growth of maize. Kernels of the genotype B73 \times LH51 were planted on 1 June 1990 in individual peat pots as described by Below and Gentry (1987). Fourteen-day-old seedlings were transplanted into a greenhouse hydroponic system, which consisted of 15-L containers filled with quartz gravel. To implement the N-form treatments, one half of the plots received 216 mg L^{-1} N as a 50:50 ratio of NH_4^+ -N: NO_3^- -N, while the other half was supplied with 216 mg L^{-1} NO_3^- -N. For a detailed description of the other nutrients in the hydroponic media, see Below and Gentry (1987). For both N-form treatments, 2L of fresh fertilizer solution was applied daily onto the gravel, and flowed through the container via gravity. The excess fertilizer solution which drained from the container was collected in a ribbed drip pan and manually recirculated every 5 to 6 h. At each recirculation, the pH was adjusted to $5.5 \pm .5$ by titration with dilute acid or base.

Plants were harvested at the V7 and R1 stages, and analyzed for dry weight and N content parameters as described by Heberer et al. (1985) and Swank et al. (1982), with the additional measurement of root dry weight. Tissue from root tips (1 mm of abaxial tissue) and xylem exudate (0.5 mL sap collected for 10 minutes from stem tissue decapitated 3 cm above ground level) were collected at the V7 and R1 stages, and analyzed for cytokinin. Extraction, purification, and determination of zeatin + zeatin riboside concentrations were performed by use of a commercial enzyme-linked immunosassay kit (DDS Analytical Systems, Copenhagen, Denmark), corrected for nonspecific binding (Hansen et al., 1984). Data from the hydroponic experiment were analyzed utilizing a randomized complete block design with four replicates at each sampling date.

Results and discussion

Field and gravel hydroponic studies were conducted to evaluate the effect of exogenous cytokinin and N form on plant productivity and yield. For the field study, analysis of soil extracts

before the application of fertilizer N revealed a moderate level of residual soil N in both years (Fig. 1). After the application of N-form treatments, analysis of soil extracts confirmed that the NO_3^- plots contained predominantly NO_3^- -N in both years (Fig. 1). In contrast, the $\text{NH}_4^+ + \text{NO}_3^-$ plots contained a higher percentage of the soil N as NH_4^+ -N for the majority of the growing season (Fig. 1). Based on the analysis of soil extracts, distinct N-form treatments were established and maintained in both years.

For the control (i.e. untreated) plants, $\text{NH}_4^+ + \text{NO}_3^-$ nutrition increased grain production by 11% (Table 1), similar to results of Below and Gentry (1987). The increased productivity of untreated $\text{NH}_4^+ + \text{NO}_3^-$ plants was associated with greater dry-matter partitioning to the grain, and not with an increase in shoot dry matter (Table 1). Similar to previous reports (Below and Gentry, 1987; Schrader et al., 1972), $\text{NH}_4^+ + \text{NO}_3^-$ nutrition of untreated plants increased N accumulation in whole shoots and grain compared to plants receiving predominantly NO_3^- (Table 1). However, when sprayed with cytokinin, N content of the whole shoot or grain was not increased by $\text{NH}_4^+ + \text{NO}_3^-$ nutrition. Although speculative, the lack of increase in N accumulation for cytokinin-treated plants may indicate that cytokinins can mimic or replace the beneficial aspects of $\text{NH}_4^+ + \text{NO}_3^-$ nutrition associated with enhanced N accumulation.

The increased grain production of untreated plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$ was associated with several changes in reproductive development. For example, the magnitude of kernel

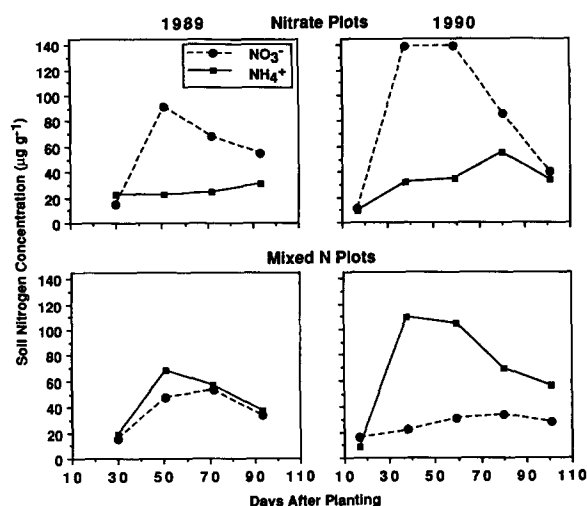


Fig. 1. Seasonal pattern of form and amount of N available within the top 25 cm of soil following application of N treatments designed to achieve soil environments of predominantly NO_3^- or as a mixture of NH_4^+ and NO_3^- in 1989 and 1990. Nitrogen treatments received a total of 224 kg N ha^{-1} , and were applied 31 days after planting (DAP). The V7 and R1 maturity stages were reached at 45 and 73 DAP in 1989 and 57 and 86 DAP in 1990.

abortion decreased when plants were supplied with $\text{NH}_4^+ + \text{NO}_3^-$ thereby increasing kernel number at maturity (Table 2). Untreated plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$ also tended to increase earshoot prolificacy, as well as the number of ovules per earshoot compared with untreated NO_3^- plants (Table 2).

Plants grown in the gravel hydroponic system exhibited developmental and morphological traits similar to those of corresponding field-grown plants, and the use of hydroponic tech-

Table 1. Effect of N-form application as predominantly NO_3^- or as a mixture of NH_4^+ and NO_3^- and cytokinin treatment on productivity parameters at physiological maturity. For the cytokinin treatment, six foliar applications of $22 \mu\text{M}$ 6-benzylaminopurine were made during vegetative growth. Values presented are averaged over the 1989 and 1990 growing seasons

Exogenous cytokinin	N-form treatment	Grain weight	Dry matter	Reduced N	Grain N	Harvest index (%)
		(g shoot ⁻¹)				
Control	NO_3^-	157	295	3.3	2.2	53.4
	$\text{NH}_4^+ + \text{NO}_3^-$	174	311	3.5	2.4	56.1
Sprayed	NO_3^-	171	300	3.3	2.3	56.9
	$\text{NH}_4^+ + \text{NO}_3^-$	169	299	3.3	2.3	56.4
LSD _{0.05}		12	NS ^a	0.2	0.1	2.5

^aNS = nonsignificant at $p < 0.05$ probability level.

Table 2. Effect of N-form application as predominantly NO_3^- or as a mixture of NH_4^+ and NO_3^- and cytokinin treatment on parameters associated with reproductive development. For the cytokinin treatment, six foliar applications of $22 \mu\text{M}$ 6-benzylaminopurine were made during vegetative growth. Values presented are averaged over the 1989 and 1990 growing seasons

Exogenous cytokinin	N-form treatment	Ovule potential plant ⁻¹	Kernel abortion (%)	Earshoot number plant ⁻¹	Kernel number plant ⁻¹
Control	NO_3^-	889	35.5	2.1	549
	$\text{NH}_4^+ + \text{NO}_3^-$	986	29.2	2.5	599
Sprayed	NO_3^-	1092	27.9	2.6	629
	$\text{NH}_4^+ + \text{NO}_3^-$	952	30.6	2.4	586
LSD _{0.05}		NS ^a	6.1	NS	44

^aNS = nonsignificant at $p < 0.05$ probability level.

niques allowed for greater precision in the delivery of specific N-form treatments. In hydroponics, $\text{NH}_4^+ + \text{NO}_3^-$ nutrition increased earshoot dry weight by 100% (Table 3), which was associated with a greater partitioning of dry matter to the shoot or increased N accumulation (Table 4). Because no differences in dry matter accumulation and partitioning, or in N content were observed at V7 (Tables 3 and 4), N form-induced alterations in plant growth and development must have occurred during the mid-to late-vegetative stages. Similar to field-grown plants, $\text{NH}_4^+ + \text{NO}_3^-$ nutrition resulted in a small, but nonsignificant, increase in the number of ovules per earshoot at anthesis (Table 3). Thus, results from both field and hydroponic studies show that $\text{NH}_4^+ + \text{NO}_3^-$ -induced increases in productivity can be attributed to an enhanced partitioning of assimilate to the developing earshoots.

For the field study, six foliar applications of $22 \mu\text{M}$ 6-benzylaminopurine (BAP) were applied during vegetative growth in an attempt to increase the supply of cytokinin to the shoot,

especially for NO_3^- -grown plants. We theorized that the increased productivity of $\text{NH}_4^+ + \text{NO}_3^-$ plants (Tables 1 and 3) was partially attributable to an increased supply of cytokinin to the developing shoot. Because supplying $\text{NH}_4^+ + \text{NO}_3^-$ has been shown to alter root morphology (Wang, 1990), and the root is the primary source of cytokinin synthesis (Simpson, 1986), the supply of cytokinin may be indirectly stimulated under $\text{NH}_4^+ + \text{NO}_3^-$ conditions. Our data provide evidence in support of this view, as cytokinin treatment of plants supplied with NO_3^- had grain yields equivalent to that of treated and untreated $\text{NH}_4^+ + \text{NO}_3^-$ plants (Table 1). The increased grain production of NO_3^- -grown plants treated with cytokinin was also attributed to enhanced dry matter partitioning to the grain, and not to an increase in shoot dry matter production (Table 1). In addition, the cytokinin application to NO_3^- plants decreased kernel abortion, thereby increasing kernel number (Table 2). Based on these data, productivity increases associated with cytokinin treatment of NO_3^- -grown plants ap-

Table 3. Effect of N-form application as NO_3^- or as a mixture of NH_4^+ and NO_3^- on vegetative and early reproductive characteristics. Values presented are for the maize genotype B73 \times LH51 grown in a gravel hydroponic system

Growth stage	N-form treatment	Shoot:root ratio (g g ⁻¹)	Shoot weight	Earshoot weight	Ovule potential plant ⁻¹
			(g plant ⁻¹)		
V7	NO_3^-	2.19	11.9	NA ^a	NA
	$\text{NH}_4^+ + \text{NO}_3^-$	2.07	13.1	NA	NA
R1	NO_3^-	1.30*	111.5	5.9*	1532
	$\text{NH}_4^+ + \text{NO}_3^-$	1.95	115.2	11.8	1799

^aNA = not applicable.

*Significant at 0.05 probability level for comparison between N-form treatment within growth stage.

Table 4. Effect of N-form application as NO_3^- or as a mixture of NH_4^+ and NO_3^- on vegetative and early reproductive N accumulation and cytokinin supply. Values presented are for the maize genotype B73 \times LH51 grown in a gravel hydroponic system

Growth stage	N-form treatment	Reduced N content			Zeatin + zeatin riboside	
		Root	Shoot	Ear	Root tip (pmol gfw ⁻¹)	Xylem exudate (pmol mL ⁻¹)
		(g plant ⁻¹)				
V7	NO_3^-	0.1	0.3	NA ^a	17.4*	4.7
	$\text{NH}_4^+ + \text{NO}_3^-$	0.1	0.4	NA	24.9	5.3
R1	NO_3^-	1.1	2.0*	0.1*	27.1	23.2
	$\text{NH}_4^+ + \text{NO}_3^-$	0.9	2.5	0.3	31.1	19.2

^aNA = not applicable.

*Significant at 0.05 probability level for comparison between N-form treatment within growth stage.

peared to be related to enhanced dry matter partitioning to the developing kernels, and decreased kernel abortion.

Although exogenous cytokinin treatment increased grain production of NO_3^- -grown plants, the role of endogenous cytokinin in $\text{NH}_4^+ + \text{NO}_3^-$ -induced increases in grain yield remains uncertain. Therefore, a gravel hydroponic study was conducted to examine the synthesis and supply of endogenous cytokinin for plants grown with $\text{NH}_4^+ + \text{NO}_3^-$ versus NO_3^- . At the V7 growth stage, the concentration of cytokinin (zeatin + zeatin riboside) contained within root tips was increased by 43% for plants supplied with both N forms (Table 4). This difference in root tip cytokinin concentration between the N-form treatments was not observed at anthesis nor was cytokinin concentration in xylem exudate altered by N-form treatment (Table 4). However, xylem exudate collected at anthesis contained higher concentrations of zeatin + zeatin riboside for both N-form treatments (Table 4), which may be reflective of larger root mass at anthesis (data not shown). Although differences in zeatin + zeatin riboside concentrations were observed for the N-form treatments, other cytokinins were not quantified in this study. It is possible that N-form treatment may interact with these forms of cytokinin to alter the productivity response of the plant.

In summary, hydroponic techniques and analysis of soil extracts for the field-grown maize plants indicated that NO_3^- plots contained predominantly NO_3^- -N, while $\text{NH}_4^+ + \text{NO}_3^-$ plots contained both NH_4^+ and NO_3^- throughout most

of the life cycle. Supplying both N forms to the plant doubled earshoot dry weight at anthesis in the hydroponic study and increased grain yield by 11% in the field study. In both cases, the enhanced productivity of $\text{NH}_4^+ + \text{NO}_3^-$ plants was associated with increased assimilate partitioning to the developing sink. Six exogenous applications of 22 μM 6-benzylaminopurine during vegetative development to plants supplied with NO_3^- -N elevated grain production to a level similar to that of $\text{NH}_4^+ + \text{NO}_3^-$ plants. In addition, the endogenous concentration of zeatin + zeatin riboside of root tip segments and xylem exudate was increased during vegetative development for plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$. Thus, these studies indicate that the increased productivity of plants supplied with $\text{NH}_4^+ + \text{NO}_3^-$ may be partly associated with an enhanced supply of endogenous cytokinin.

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