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Relationship between Nitrate Uptake, Flux, and Reduction and the Accumulation of Reduced Nitrogen in Maize (*Zea mays* L.)

I. GENOTYPIC VARIATION[1]

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ABSTRACT

The study presented here was an extension of a preceding field project concerned with changes in N metabolism of four maize hybrids during grain development. The objectives were to relate uptake, flux, and reduction of nitrate to accumulation of reduced N in growth-chamber-grown seedlings of the same four hybrids and to compare these results with those obtained in the field study.

Hybrid D took up more nitrate than the other three hybrids, primarily because of a larger root system. The correlations between total N (nitrate plus reduced N plant^{-1}) accumulated by harvest and root dry weight or shoot to root ratios were $r = +0.97$ and -0.90, respectively. Correlations with shoot dry weight were low. Although the larger root system indicates enhanced partitioning of photosynthate to the root of hybrid D, the observations made do not elucidate the role of photosynthate in increasing nitrate uptake. There was no genetic difference in partitioning of nitrate (per cent of total) among the plant parts; however, the hybrids differed in amounts of nitrate stored in stalks and midribs. Hybrids D and B accumulated more nitrate than A and C.

Although two of the hybrids (A and C) with highest nitrate reductase activity had the lowest concentrations of nitrate in all plant parts, nitrate reductase activity was not correlated with accumulation of nitrate or reduced N for the four hybrids. Uptake and flux of nitrate were not numerically related to accumulation of reduced N for the four hybrids. Among the four hybrids, nitrate flux was not associated with level of leaf nitrate reductase activity. None of the individual parameters, as measured, would serve as an index for reduced N accumulation for these four hybrids. When the hybrid pairs were compared separately, it was evident that both rate of nitrate flux and level of nitrate reductase activity affect the accumulation of reduced N by the plant.

Relative to the other hybrids, hybrid D that accumulated the most reduced N and nitrate as a 23-day-old seedling had the least reduced N in grain plus stover at maturity under field conditions. Hybrid C that had high nitrate reductase activity as a seedling had low nitrate reductase activity after anthesis under field conditions. These changes in metabolic activities with plant development and different environments illustrate the problems encountered in attempting to develop simple physiological or biochemical screening criteria useful in identifying superior cultivars at the seedling stage.

In wheat and corn, significant correlations between the estimated amount of reduced N supplied to the plant (by the *in vitro* or *in vivo* NR[3] assay) and the actual amount accumulated by the plant (2, 5, 7, 8) support the concept that nitrate reduction is the rate-limiting step in the assimilation of nitrate to reduced N. Significant correlations were found between integrated seasonal leaf NRA and grain yield, plant reduced N, and grain reduced N for maize. In these and other experiments, the correlation values were low, indicating that factors other than the level of leaf NRA affect these relationships.

Dalling *et al.* (5) showed that transport of vegetative N to the grain of wheat was one of these factors. They also found that wheat cultivars with comparable levels of NRA could accumulate different amounts of reduced N. Deckard *et al.* (6) identified one maize genotype with a relatively low NRA but a high capacity to accumulate reduced N. The fact that this genotype had a high leaf nitrate concentration suggests that the availability of nitrate to NR in the leaf, as well as the level of enzyme, may affect the rate of nitrate reduction (3). This possibility is supported by (*a*) the stimulation of the *in vivo* NR assay with nitrate (10), and (*b*) the correlation found between nitrate uptake and accumulation of reduced N in several maize genotypes (4).

Although nitrate flux to the leaves of maize regulates the level of NR (21), it has not been shown that nitrate flux and NR are directly related among genotypes. Since the nitrate flux provides both substrate for and inducer of NR, genotypic differences in the partitioning of nitrate and/or responsiveness of the induction mechanism to nitrate could explain the discrepancy observed for the one genotype in the study by Deckard *et al.* (7).

The study presented here was an extension of a preceding field project that utilized the same four maize hybrids (19). The field study was concerned with changes in N metabolism (enzymes and N components) in various plant parts during grain development. The objectives of the current study were: (*a*) to relate nitrate uptake, nitrate flux, shoot to root partitioning of nitrate reduction, and NRA to the accumulation of reduced N by the plants during early vegetative growth; and (*b*) to compare these results with seedlings to those obtained in the preceding field study.

MATERIALS AND METHODS

PLANT CULTURE

Maize (*Zea mays* L.) hybrids B37 × B73 (A), Mo17 × H95 (D), C123 × B14A (C), and B37 × H96 (B) were selected and paired (A-D and C-B) for comparison based on preceding work (ref. 19 and unpublished data). Hybrids A and B had high levels and hybrids C and D had low levels of NRA (leaf, postanthesis) under

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[3] Abbreviations: NR, nitrate reductase; NRA, nitrate reductase activity.

field conditions. Kernels were germinated in the dark at 28 C on paper towelling soaked in 10^{-4} M $CaSO_4$. After 3 days, 42 uniform and vigorous seedlings of each genotype were transferred to three 5-liter plastic pots that contained 4.5 liters continuously aerated nutrient solution of the following composition: 0.125 mM KH_2PO_4, 0.31 mM $CaCl_2$, 0.31 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.63 mM K_2SO_4, 0.5 mM $MgSO_4$, 9 μM $FeSO_4$, and 0.08 mM Fe as Chel-330 (Ciba-Geigy Corp., Greensboro, N. C.) and a full complement of micronutrients (9). The pH was adjusted to 4.0 with H_2SO_4. Each plant was held in position in the lid of the pot by a split plastic tubing collar and cotton wool. The hybrid pairs were grown in separate growth chambers, with similar environmental conditions (16-h photoperiod, 450 μE m^{-2} s^{-1}, and light and dark temperatures of 30 and 24 C, respectively). Six days after planting, the plants were thinned to eight/pot to achieve maximum uniformity in size. Concurrently, the composition of solutions were changed by increasing the macronutrient concentrations 2-fold and the iron concentrations 4-fold. A resin-exchange column (12) was also added to each pot to maintain the pH at 4.0.

When the plants were 11 days old, the concentrations of the macronutrients salts were doubled. Thereafter, the solutions were changed every other day and maintained at 4.5 liters by periodically adding deionized H_2O. Plants were removed at the 15-day-old stage so that four plants of uniform size remained in each pot.

Hybrid pair A and D was harvested at the 23-day-old stage and the C and B pair was harvested at the 25-day-old stage. Consequently, most comparisons will be made between the two pair of hybrids (A-D and C-B). The staggered harvest was employed because of the number of assays involved. After 6 h illumination, shoots were excised at the first leaf node, stored in plastic bags, and placed in ice (3 C) prior to weighing and assay. After collecting the xylem sap from each plant, the stumps were excised from the roots and stored with their corresponding shoots. The roots from each pot were washed thoroughly with distilled H_2O, blotted dry with paper towels, and stored in plastic bags in ice before weighing and assay. Plant parts and xylem exudate of the four plants from one pot were composited to make a single sample.

NITRATE UPTAKE

Nitrate uptake was measured by disappearance of nitrate from the nutrient media over a 24-h period just prior to harvesting the plants.

TRANSPIRATION MEASUREMENTS

Transpiration was determined by the change in weight of nutrient pots with plants over a 3-h period just prior to harvesting the plants. Comparable pots, with nutrient solution but without plants, were used to correct for nontranspirational evaporation. Transpiration rates were expressed as ml g^{-1} leaf fresh weight h^{-1}.

NITRATE CONTENT OF XYLEM SAP

Immediately after the shoots were excised, the stumps were blotted dry and the first 25 μl exudate from the cut surface of each stump was collected, as previously described (22). Nitrate flux was calculated by multiplying transpiration rate by xylem nitrate concentration (μmol NO_3^- g^{-1} leaf fresh weight h^{-1}).

PREPARATION OF CELL-FREE EXTRACTS

NR was extracted from leaf blades as previously described (14). All leaves from the four plants in each pot were deribbed, composited, and chopped into 10-mm^2 sections. Duplicate aliquots (3 g) from each sample were homogenized with a VirTis 45 homogenizer (VirTis Research Equipment, Gardiner, N. Y.) for 2 min at half-line voltage, using a ratio of 1:10 (w/v) of extraction medium. The crude homogenates were filtered through four layers of cheesecloth and centrifuged at 30,000*g* for 15 min, and the supernatants were used for NR and nitrate assays. Samples used for all NRA assays were representative of the entire leaf canopy or root system.

The midribs, stalks (includes leaf sheaths), and roots were processed separately, as described for leaves with the following exceptions. Portions (5 to 10 g) from each sample were homogenized for 3 min in distilled H_2O (1:100, w/v) using a Waring Blendor. The homogenates were filtered through two layers of cheesecloth and clarified by centrifugation, and the supernatant fractions were used for the nitrate assay. Other portions were used for dry-weight and reduced-N assays.

NITRATE REDUCTASE

In Vitro. The procedure described by Scholl *et al.* (20) was used.

In Vivo. The leaf *in vivo* NR assay was the same as previously described (2), except 0.04% (v/v) Neutronyx 600 (nonionic surfactant, Onyx Chemicals, Jersey City, N. J.) was used in the assay medium. The root *in vivo* assay was essentially the same as that for the leaf, except 2.5% 1-propanol was used as a surfactant in the assay medium, and the ratio of tissue to medium was 1:10 (w/v).

DRY WEIGHT, REDUCED N, AND NITRATE

Plant samples were dried for 3 days in a forced-air oven at 60 C. Total reduced N of the plant parts was determined in the powdered samples as described (2). Nitrate was measured as described (11).

VERMICULITE CULTURE

In the first experiment (age effect), 25 kernels of a given hybrid were planted in Vermiculite in a plastic pan (30 × 15 × 15 cm) and thinned to 12 uniform plants 7 days after planting. Three replicate pans were used for each hybrid. The kernels and plants were subirrigated (pans had perforated bottoms) daily with a modified Hoagland No. 1 solution (9). Nitrate was 7.5 mM and Ca^{2+} and K^+ were made to full strength by adding $CaCl_2$ and K_2SO_4, respectively. The iron and micronutrient concentrations were as previously described and the pH was adjusted to 4 with H_2SO_4. The hybrid pairs were grown in separate growth chambers. Plants were harvested (three plants/sample, and triplicate samples for each hybrid) at different ages and parts were assayed by procedures previously described. The same procedures were used for the second experiment (time of sampling) except that pots (19 cm diameter × 15 cm) were used as containers, 15 plants were grown in each pot, and all plants were grown in a single growth chamber. The plants were 12 days old when harvested. For both Vermiculite experiments, the growth-chamber environments were as previously described. Plant vigor and growth in the Vermiculite were superior to those in the nutrient solution cultures.

RESULTS

Dry Weight and Reduced N Accumulation. For the A-D hybrid pair, hybrid D accumulated more total plant dry matter than A and had a greater percentage of its total dry weight in the root (Table I). The increased root mass of hybrid D implies a greater partitioning of photosynthate to the root. For the hybrid pair C-B, there were no significant differences in either total dry matter accumulation or partitioning between root and shoot.

There was no difference in the concentration of reduced N in the shoots of hybrids A and D, whereas hybrid C had a higher concentration than B (Table II). Hybrid D accumulated more reduced N/shoot than hybrid A because of its greater dry weight.

Table I. *Dry Weights of Shoot and Root of Four Maize Hybrids*

Hybrids A and D were 23 days old and C and B were 25 days old at harvest. The dry weight to fresh weight ratios were not significantly different.

Hybrid	Plant Part Dry Wt			Shoot/Root Dry Wt Ratio
	Shoot	Root	Total	
		g		
A	1.77 ± 0.10	0.52 ± 0.06	2.29 ± 0.12	3.39 ± 0.20
D	2.38 ± 0.04	0.82 ± 0.07	3.20 ± 0.11	2.90 ± 0.15
C	2.16 ± 0.20	0.55 ± 0.04	2.70 ± 0.22	3.96 ± 0.20
B	2.14 ± 0.23	0.54 ± 0.05	2.68 ± 0.28	3.96 ± 0.07

Table II. *Concentration and Accumulation of Reduced N by Shoots and Roots of Four Maize Hybrids*

Hybrid pair A-D was 23 days old and hybrid pair C-B was 25 days old at harvest.

Hybrid	Reduced N			
	Shoot		Root	
	mg g^{-1} dry wt	*mg $plant^{-1}$ part*	*mg g dry wt^{-1}*	*mg $plant^{-1}$ part*
A	39.0 ± 1.5	68.9 ± 2.8	32.5 ± 1.9	17.0 ± 2.0
D	39.5 ± 2.0	93.1 ± 4.3	32.5 ± 2.0	24.8 ± 1.8
C	32.4 ± 1.2	70.1 ± 2.5	21.8 ± .90	11.9 ± 1.4
B	29.4 ± 1.6	62.9 ± 1.9	21.4 ± .95	11.5 ± 1.2

Table III. *Leaf and Root NRAs of Four Maize Hybrids*

Samples were taken at time of harvest and were representative of the entire leaf canopy and root system.

Hybrid	Leaf Blade		Root *in Vivo*
	In vitro	*In vivo*	
	μmol NO_2^- h^{-1} g^{-1} fresh wt		
A	15.0 ± 0.15	2.48 ± 0.06	1.18 ± 0.03
D	10.4 ± 0.08	1.99 ± 0.11	0.87 ± 0.02
C	14.2 ± 0.35	2.66 ± 0.18	0.99 ± 0.03
B	11.8 ± 0.27	1.83 ± 0.10	0.93 ± 0.04

Hybrid D and C accumulated more reduced N/shoot than did their paired counterparts, hybrids A and B, respectively. With respect to root reduced N for the two hybrid pairs, the only difference was that hybrid D accumulated more reduced N than hybrid A primarily because of larger root weight.

Genotypic Differences in NRA. Leaf NRA, assayed *in vivo* or *in vitro*, differed significantly between the hybrids of each pair (A > D; C > B), but differences of root *in vivo* NRA were observed only between hybrids A and D (Table III). Of the four hybrids, D had the lowest NRA/g root, an observation consistent with the observation that low root NRA permits greatest root growth (16). For each hybrid pair, the relative rankings with respect to level of leaf NRA was the same with both assays; however, greater differences in NRA were obtained with the *in vitro* than the *in vivo* assay for genotypes A and D. For both hybrid pairs, these differences could be due to *in vitro* assay problems, such as enzyme stability (21), to the presence of inhibitors (6, 23), or to the fact that availability of reductant limits the *in vivo* reduction (14).

The high level of leaf blade NRA of hybrid C (Table III) was unexpected as this hybrid had been shown to have low levels of NRA when leaves were assayed during the reproductive phase (19).

Nitrate Uptake and Flux to Leaves. There was no difference in the nitrate uptake/g root weight between the hybrid pairs (Table IV). However, because hybrid D had a larger root system than A, nitrate uptake/per plant differed for these two genotypes.

Table IV. *Differences in Concentration of Nitrate of Xylem Exudate and Rates of Transpiration, Nitrate Uptake, and Flux of Four Maize Hybrids*

Measurements made at or near time of harvest when hybrids A and D were 23 days old and C and B were 25 days old.

Hybrid	Transpiration/g Leaf Fresh Wt	Concn of Xylem Exudate	Nitrate Uptake		Flux/g leaf fresh wt
			Per g root dry wt	Per plant	
	ml h^{-1}	*μmol ml^{-1}*	*μmol h^{-1}*		
A	0.60 ± 0.01	10.3 ± 0.6	92 ± 6	49 ± 13	6.2 ± 0.5
D	0.59 ± 0.03	14.8 ± 0.2	90 ± 4	74 ± 5	8.7 ± 0.4
C	0.72 ± 0.02	15.2 ± 0.3	140 ± 4	76 ± 4	10.9 ± 0.1
B	0.67 ± 0.02	16.1 ± 0.3	139 ± 4	75 ± 5	10.8 ± 0.1

Table V. *Concentration of Nitrate in Various Plant Parts of Four Maize Hybrids Grown on Nutrient Culture*

Hybrid pairs A-D and C-B were harvested when 23 and 25 days old, respectively. Values in parentheses are percentages of total nitrate content plant part.

Hybrid	Nitrate Concn.			
	Leaf Blade	Midrib	Stalk	Roots
	μmol g^{-1} fresh wt			
A	10 ± 0.5 (6)	56 ± 2 (19)	56 ± 1 (45)	44 ± 2 (30)
D	16 ± 0.7 (6)	90 ± 3 (21)	74 ± 2 (47)	43 ± 1 (26)
C	11 ± 0.4 (5)	47 ± 5 (17)	63 ± 3 (49)	43 ± 1 (29)
B	15 ± 0.1 (7)	62 ± 4 (20)	73 ± 2 (51)	42 ± 1 (24)

Comparison of nitrate uptake/plant and nitrate flux for both hybrid pairs shows that the two processes are related but does not necessarily mean that they are causally related.

Nitrate Accumulation. Nitrate concentrations in the roots were the same for all four hybrids (Table V), although root NRA (Table III) and flux rate (Table IV) of the hybrids differed. This indicates that root nitrate concentration may be determined by the nitrate concentration of the external medium and equivalent to the fixed nitrate pool identified by Ashley *et al.* (1).

For both hybrid pairs, the concentration of nitrate in the storage organs (midribs and stalks), as well as in the leaf blades, was lower for the hybrids A and C that had higher levels of leaf blade NRA than their respectively paired counterparts (Tables III and V). This indicates that leaf-blade NRA level was a factor affecting nitrate accumulation. This is especially valid for hybrid pair C-B that had comparable rates of nitrate uptake and flux and accumulated comparable amounts of N (nitrate plus reduced N). For the other pair, the lower rate of nitrate uptake and flux of hybrid A could be responsible in part for the difference in nitrate accumulation between the A-D pair.

There was no apparent effect of nitrate uptake or amount of total nitrate accumulated on the partitioning (per cent of total) of nitrate among the various plant parts of the four hybrids.

Vermiculite Cultures. Supplementary experiments were conducted with Vermiculite-grown plants: (*a*) to confirm the observation that hybrid C had higher levels of NRA than the other hybrids when plants were sampled in the seedling (vegetative) stage and (*b*) to determine the effect of seedling age and time of sampling on the relative rankings of the four hybrids with respect to NRA and concentrations of midrib nitrate and shoot-reduced N.

The Vermiculite studies confirmed that, during early vegetative development, hybrid C had higher levels of NRA than the other hybrids (Figs. 1 and 2). In previous studies with field-grown plants (ref. 19 and unpublished data), the ranking of these four hybrids

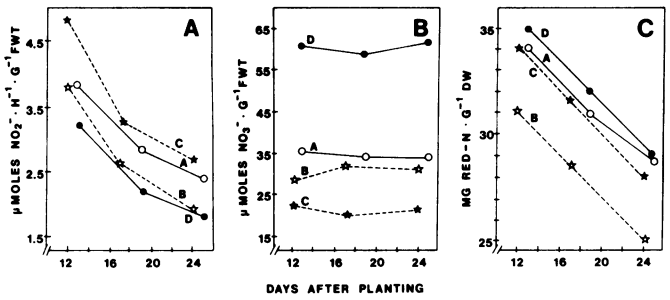

FIG. 1. Effect of plant age on *in vivo* NRA, midrib NO_3^- concentration, and shoot reduced N concentration in four maize hybrids.

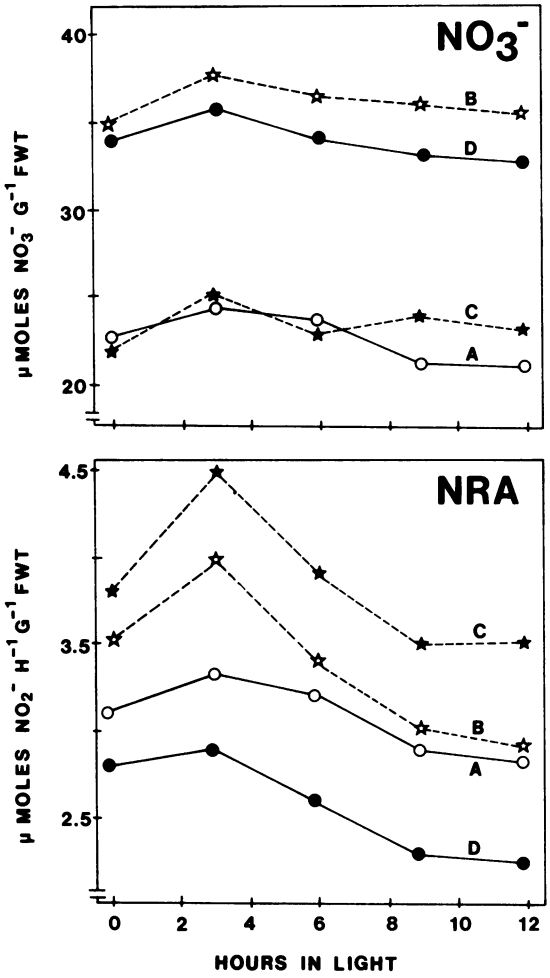

FIG. 2. The variation of *in vivo* NRA and midrib nitrate concentrations of four maize hybrids during 12 h illumination. Plants were 12 days old at harvest.

for postanthesis leaf-blade NRA (*in vivo* assay) was consistently B = A > C = D (3 years observations). In these studies, the rankings (*in vivo* assay) were C = A > D = B (Table III) or C = A > B = D (Fig. 1). The classification change of hybrid C from a "high" to a "low" NRA type with plant development (vegetative to reproductive stage) was not anticipated. Using maize plants grown under comparable conditions, Warner (25) had found that relative rankings with respect to NRA did not change with plant development. However, only a few genotypes were surveyed.

With respect to the hybrid pairs, there was no change in relative ranking of the hybrids for NRA, concentration of midrib nitrate, or reduced N as a function of seedling age or time of sampling (Figs. 1 and 2; Tables II, III, and V).

With respect to all hybrids, the value and ranking for NRA (Fig. 1) was nearly identical with previous results (Table III) obtained with leaves of comparable age. Although hybrid B initially ranked higher in NRA than did hybrid D, it lost activity faster as the plant aged (Fig. 1). Consequently, by the end of the experiment, its activity was equal to that of hybrid D and lower than that for hybrid A. Relative rankings of the hybrids with respect to shoot-reduced N were identical in both experiments (Fig. 1; Table II); however, with material of comparable age, the values were much lower for the Vermiculite-grown plants. The midrib nitrate values were also lower in the Vermiculite-grown plants (Fig. 1; Table V). These lower values can be attributed to growth dilution (Vermiculite plants were larger) or/and differences in supply of nitrate (Vermiculite cultures supplied less nitrate). In both experiments, hybrid D accumulated the most midrib nitrate and hybrid C accumulated the least (Fig. 1; Table V). Recognizing the small differences in nitrate values of hybrids A and B, the altered rankings of the two hybrids in these experiments is considered meaningless.

Sampling time had no effect on the relative rankings of the four hybrids with respect to NRA (Figs. 1 and 2). (Values for hybrids A and B are identical in leaves from 12-day-old material sampled after six h of illumination.) Both NRA and midrib nitrate exhibited cyclic patterns, with maxima after 3 h illumination, which may indicate that cyclic nitrate flux (15, 24) may be affecting the midmorning increase in NRA (22). Hybrids C and B exhibited greater fluctuations in NRA than did A and D. The values and rankings for midrib nitrate concentrations of the four hybrids (Fig. 2) differed from those obtained in previous experiments. Hybrids A and D accumulated much less and hybrids C and B accumulated slightly more nitrate than in previous experiments (Fig. 1; Table V). The lower accumulation of nitrate by hybrids A and D and the lower levels of NRA of all hybrids are consistent with the decreased nitrate supply (more plants/pot, smaller volume of Vermiculite; see "Materials and Methods. Although the reason for the increase in midrib nitrate by hybrids C and B is not known, it is speculated that hybrids B and D are more effective at taking up nitrate than A and C under these conditions (lower concentration of nitrate in the rooting medium).

DISCUSSION

Comparisons of data for hybrid pair A and D indicate that accumulation of reduced N was more dependent upon nitrate uptake and flux than upon the amount of NRA/plant (Table VI). For the other pair, hybrid C with more NRA accumulated more reduced N and stored less nitrate than hybrid B, although both hybrids had comparable uptake and flux of nitrate. For hybrids C and B, the equality in uptake and flux is associated with equality of total N (reduced N plus nitrate N)/plant. These data show that both flux and NRA affect the accumulation of reduced N.

Comparisons of data for all hybrids (disregarding harvest age)

Table VI. *A Comparison of Rates of Nitrate Uptake, Flux, and Reduction to Contents of Nitrate and Reduced N of Four Maize Hybrids*

Hybrid pairs A-D and B-C were harvested at 23 and 25 days after planting, respectively. Nitrate reduction was measured with *in vivo* assays of leaf blades and roots.

Hybrid	Nitrate			Component Contents	
	Uptake	Flux	Reduction	Nitrate	Reduced N
	μmol plant^{-1} h^{-1}			*mmol plant^{-1}*	
A	48.6	46.6	30.3	1.39	6.14
D	74.2	73.6	30.7	2.43	8.42
C	76.1	74.9	29.5	1.69	5.86
B	75.1	73.4	22.6	1.93	5.31

show that nitrate uptake, flux, and reduction (as measured) are not numerically related to accumulation of reduced N (Table VI). Differences in plant age at time of harvest may be one factor that precluded correlations among these parameters. For example, for each hybrid pair, the rates of nitrate uptake and flux are consistent with the accumulation of total N (reduced N plus nitrate) content of the plants. Relative rates of plant growth, differences in diurnal patterns of uptake and flux, and differences in requirements for nitrate for induction and assimilation are other factors that could affect these relationships.

Of all possible comparisons of the five parameters (Table VI), only nitrate uptake *versus* flux was correlated ($r = +0.99$). The occurrence of such a relationship would be useful; however, additional measurements with more genotypes and variations in uptake rates are needed to establish validity. Among the four genotypes, NRA was not correlated with nitrate flux when the data were expressed either per plant (Table VI) or per unit weight basis (Tables III and IV). That NRA and nitrate flux were closely associated for a given maize genotype (22), but not among genotypes, is not surprising as it seems probable that genetic differences may exist in systems that affect induction, stability, and activity of NR.

The correlations between total N (nitrate plus reduced N) accumulated by harvest and root dry weight and shoot to root ratio were $r = +0.97$ and -0.90, respectively. The correlations with shoot dry weight were low. Because of the limited number of observations, the validity of these correlations may be questioned. Those correlations are consistent with the observations with tobacco, cotton, and soybean plants that "rate of nitrogen uptake is co-equal with rate of root growth" (17, 18). The larger root of hybrid D, relative to the other hybrids, is a reflection of a greater diversion of photosynthate to the root of hybrid D. According to the interdependence concept (17, 18), the photosynthate is required for both root growth and nitrate uptake. Other investigators hold that it is the characteristics of the root mass (length, surface, etc.) that are responsible for the enhanced uptake (13).

Under field conditions, the reduced N content of hybrids A, D, C, and B were 265, 241, 290, 291 mmol/plant (grain plus stover at maturity), respectively (19). Hybrid D, that had the highest amount of reduced N at the seedling stage, had the least at maturity. The large root mass and associated high levels of uptake, flux, and reduction observed for hybrid D growth chamber seedlings apparently either were not achieved or were not maintained under field conditions (19). Under field conditions, hybrid C was consistently classified as a "low NRA" type when leaves were assayed after anthesis; however, during vegetative development under growth-chamber conditions, hybrid C was found to be a "high NRA" type. These observations illustrate the problems encountered in attempting to develop simple physiological and biochemical screening criterion useful in identifying superior cultivars at the seedling stage.

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