SHOOT AND ROOT NITRATE REDUCTASE ACTIVITY AS RELATED TO N ACCUMULATION AND ASSIMILATION IN 16 CORN INBREDS[1]

(HUBUNGAN ANTARA AKTIVITAS NITRAT REDUKTASE PADA AKAR DAN TAJUK DENGAN AKUMULASI DAN ASIMILASI N PADA 16 INBRED JAGUNG)

Oleh

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Abstract: The relationships between nitrate reductase activity (NRA) in the shoots and roots, NRA versus net nitrate uptake, and NRA versus accumulation of nitrate and reduced N were studied in 15-day-old greenhouse-grown seedlings of 16 corn (*Zea mays* L.) inbreds. The distributions of NRA, total N (TN), nitrate, and reduced N (RN) between roots and shoots were determined. The observed genotypic differences in accumulated N per plant were related to differences in root size, but factors other than root size also affected nitrate absorption. Results of the present study are comparable to earlier results obtained with 12-week-old plants. Both studies indicate that selection for genotypic variation in maize inbreds for uptake or accumulation of nitrate is possible. On the average, root NRA was 10.0% and 3.0% of shoot NRA on a gram fresh weight basis and on a per plant basis, respectively. These data indicate that shoot NRA plays a greater role in N assimilation in corn seedlings than does root NRA. A positive relationship was observed in the 16 inbreds between root and shoot NRA as indicated by a highly significant correlation ($r = 0.786^{**}$) based on a gram fresh weight basis. When total NRA per shoot was compared to total NRA per root, the correlation was also highly significant ($r = 0.787^{**}$). The data suggest that root and shoot NR are both induced in parallel when high levels of nitrate are provided to corn seedlings. Corn genotypes with high shoot and root NRA also had high TN ($r = 0.772^{**}$ and $r = 0.727^{**}$, respectively). On a per plant basis, genotypes with high NRA also had high TN and RN ($r = 0.775^{**}$ and $r = 0.781^{**}$, respectively).

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Ringkasan: Hubungan antara aktivitas nitrat reduktase (NRA) pada tajuk dan akar, NRA versus pengambilan nitrat, dan NRA versus akumulasi nitrat dan N tereduksi telah diteliti pada 16 inbred jagung yang berumur 15 hari yang ditumbuhkan di dalam rumah kaca. Untuk itu dihitung distribusi NRA, N total (TN), nitrat, dan N tereduksi (RN) pada akar dan tajuk. Perbedaan genotipe yang diamati pada N terakumulasi per tanaman berhubungan dengan perbedaan pada ukuran akar, namun faktor-faktor lain di samping ukuran akar mempengaruhi juga absorpsi nitrat. Hasil penelitian ini sesuai dengan hasil penelitian sebelumnya yang diperoleh pada tanaman berumur 12 minggu. Kedua studi menunjukkan bahwa seleksi keragaman genotipik mungkin dilakukan untuk pengambilan dan akumulasi nitrat pada inbred jagung. Secara rerata, NRA akar adalah 10.0% dan 3.0% dari NRA tajuk, masing-masing berdasarkan gram berat basah dan per tanaman. Data tersebut menunjukkan bahwa NRA tajuk mempunyai peranan lebih besar daripada NRA akar dalam asimilasi N pada bibit jagung. Terdapat hubungan positif antara NRA akar dan NRA tajuk pada 16 inbred jagung yang diteliti seperti ditunjukkan oleh korelasi yang sangat nyata ($r = 0.786^{**}$) berdasarkan gram berat basah. Apabila NRA total per tajuk dibandingkan dengan NRA total per akar, maka korelasinya juga sangat nyata ($r = 0.787^{**}$). Data menunjukkan bahwa nitrat reduktase pada akar dan batang dipengaruhi secara paralel apabila nitrat dalam jumlah besar diberikan pada bibit jagung. Genotipe jagung dengan NRA tajuk dan akar yang tinggi mempunyai juga TN yang tinggi (masing-masing $r = 0.772^{**}$ dan $r = 0.727^{**}$). Dengan dasar per tanaman, genotipe dengan NRA tinggi mempunyai juga TN dan RN tinggi (masing-masing $r = 0.775^{**}$ dan $r = 0.781^{**}$).

INTRODUCTION

Nitrate reductase (NR) is the rate limiting enzyme in the assimilation of nitrate to reduced N (3). The site of nitrate reduction in the plant is very important because reduction requires considerable energy. Schrader and Thomas (25) suggested that nitrate reduction in the dark or in non-green tissue is about twice as expensive as reduction in illuminated green tissue. Although more nitrate reduction occurs in shoots than in roots of most plant species, roots are a significant site of reduction (3, 16, 17). Ivanko and Ingversen (12) showed that

nitrate is assimilated into amino acids and protein in corn (*Zea mays* L.) roots.

The level of nitrate reductase activity (NRA) varies considerably within a species (8, 16, 24, 28). Pate (16) showed that species with high root NRA transported high levels of reduced N to shoots whereas species with low root NRA transported more nitrate as compared to reduced N. Thus, it seems possible that genotypes with low root NRA might have high shoot NRA and vice versa. However, few or no comparisons have been made of genetic variability of root and shoot NRA within a species, and hence comparisons are difficult to make (14).

In an earlier study (Schrader, unpublished), significant differences in leaf NRA were observed among 14 inbred lines of corn. Several of those inbreds were among the 16 inbreds chosen for the present study. The present study was conducted to examine the relationship between (a) NRA in the shoots and roots of 16 inbreds of corn seedlings, (b) NRA versus net nitrate uptake, and (c) NRA versus accumulation of nitrate and reduced N.

MATERIALS AND METHODS

Plant Culture

The following 16 corn (*Zea mays L.*) inbreds were used: A619, W117, W182E, W182B, W182BN, W153R, A632, C123, Oh43, A632, W59M, W536, W729D, W627C, W438, and W64A. Corn seeds were placed in running tap water for 4 hours and then planted on June 23, 24, and 25, 1979, in plastic dishpans (35 x 30 x 15 cm) containing equal volumes of vermiculite and peat moss. The plants were watered twice a day with full strength modified Hoagland solution (7). The pans had holes in the bottom so that excess solution would drain into another plastic dishpan placed underneath. Each plastic pan contained 20 plants each of two inbreds.

Three replications of each genotype were placed in an air-conditioned greenhouse in a completely randomized design.

The maximum day temperature in the greenhouse during the growth period varied from 25 to 33°C. The minimum night temperature ranged from 15 to 21°C. The photosynthetic photon flux density at the top of the plant canopy at 1200 on a clear day (1 July) was approximately 105 nE $cm^{-2} sec^{-1}$.

The seedlings were harvested 15 days after emergence between 0900 and 1100. Each plant was separated into shoot and root fractions. Fresh weight of each fraction was recorded. The shoot and root fractions were cut into 5 mm sections, weighed and subsampled for enzyme assays. The remaining shoot and root fractions were dried (70°C) for total N (TN) and nitrate analyses.

Extractions and Assay of NRA

Shoot and root material was ground with a VirTis Model 60K homogenizer (1.0 min at 48,000 rpm). NRA was extracted by homogenizing 1 to 2 g of minced plant fractions in 6 ml of extraction buffer with casein (3% w/v) added to extraction media as described by Schrader *et al.* (23) to prevent the decay of NRA for several hours and to increase its activity. All extraction operations were performed on ice. NR extracts were assayed in triplicate as described by Hageman and Hucklesby (11). Phenazine methosulfate (PMS) was used to improve the colorimetric assay (22). Enzyme activity was expressed as umoles product formed per hr per g fresh weight.

Analysis of Nitrogenous Fractions

Dried tissue was assayed for TN by a modified microKjeldahl method (5) and for nitrate with the salicylic acid procedure of Cataldo *et al.* (6). Concentration of reduced N (RN) was calculated by subtracting nitrate from TN concentration.

Statistical Analysis

Data were analyzed by an analysis of variance. A Duncan's new multiple range test was conducted to determine specific differences among genotypes.

RESULTS

Fresh Weight

Significant differences in fresh weight of shoots, roots, and total plant were observed in 16 inbreds (Table 1). Significant differences in dry weight were also observed (data not shown). W739A, W59M, W536, W729D, W627C, and W64A were significantly higher in shoot fresh weight than W117, W153R, W182BN, A632, and C123. W739A and W59M were significantly higher in root fresh weight than A619, W117, W182E, and C123. Total plant fresh weight in W739A, W59M, W627C, W729D, W64A, W536, and A619 was significantly higher than in W117, W153R, W182BN, and C123. The shoot root ratios for fresh weight varied from 1.94 to 4.99 with a mean of 3.41.

Nitrate Reductase Activity

NRA in shoots, roots, and total plant differed significantly among genotypes (Table 2). NRA in shoots ranged from 1.90 to 9.02 umoles NO_2^- produced per gram fresh weight per hour, whereas root NRA ranged from 0.22 to 0.76 umoles NO_2^- reduced per gram fresh weight per hour. Shoot NRA was significantly higher in W536 than in all other genotypes. A632 and W117 were significantly lower in shoot NRA than were nine other genotypes. Root NRA was also highest in W536 and significantly higher than in 11 other genotypes. On the average, root NR was 10.0 % of shoot NR on a gram fresh weight basis.

Table 1. Genotypic Comparisons of Shoot, Root, and Plant Fresh Weight of 15-day-old Corn Seedlings*

Genotype		Shoot	Root	Plant
		..... g fresh weight		
1.	A619	7.03bcd	1.41c	8.44abc
2.	W117	4.92de	1.52bc	6.44d
3.	W182E	5.68cde	1.59bc	7.27cd
4.	W182B	5.79cde	1.76abc	7.55cd
5.	W153R	4.63de	1.97abc	6.60d
6.	W182BN	4.07e	2.10abc	6.17d
7.	A632	5.26de	2.03abc	7.27cd
8.	C123	4.70de	1.47bc	6.19d
9.	Oh43	6.05cde	2.17abc	8.22bcd
10.	W739A	10.60a	2.67a	13.26a
11.	W59M	9.55ab	2.56a	12.11a
12.	W536	8.31abc	2.22abc	10.53abc
13.	W729D	8.81ab	2.38ab	11.19ab
14.	W627C	10.56a	2.29abc	12.85a
15.	W438	6.14cde	1.80abc	7.94bcd
16.	W64A	8.24abc	2.37 ab	10.61abc

*Values in a column followed by the same letter are not significantly different at the 5% level using Duncan's new multiple range test.

Table 2. Partitioning of Nitrate Reductase Activity Between Shoots and Roots of 16 Corn Inbreds*

	Genotype	NRA		NRA		
		Shoot	Root	Shoot	Root	Plant
		umoles NO_2^- gfw^{-1} hr^{-1}		umole NO_2^- hr^{-1}/organ		
1.	A619	5.72bcde*	0.49bcde	39.70bcd	0.71 cd	40.41bcd
2.	W117	1.93g	0.24f	9.67e	0.36d	10.03e
3.	W182E	3.56efg	0.32def	20.20de	0.46d	20.66de
4.	W182B	5.39bcde	0.51bcde	30.13cde	0.86cd	30.99cde
5.	W153R	5.23bcde	0.56abcd	23.84de	1.07cd	24.91de
6.	W182BN	2.09fg	0.26ef	8.60e	0.53d	9.13e
7.	A632	1.90g	0.23f	10.22e	0.49d	10.71e
8.	C123	3.84efg	0.35def	18.18de	0.52d	18.70de
9.	Oh43	6.55b	0.65abc	38.89bcd	1.41abc	40.30bcd
10.	W739A	4.90bcde	0.65abc	52.60abc	1.76a	54.36abc
11.	W59M	6.00bcd	0.42cdef	59.30ab	1.08abcd	60.38ab
12.	W536	9.02a	0.76a	75.85a	1.69ab	77.54a
13.	W729D	4.15cdef	0.41cdef	37.24bcd	1.00bcd	38.24bcd
14.	W627C	6.33bc	0.69ab	65.67a	1.62ab	67.29a
15.	W438	3.67efg	0.32def	22.76de	0.58d	23.34de
16.	W64A	2.61fg	0.22f	23.01de	0.52d	23.53de

*Values in a column followed by the same letter are not significantly different at the 5% level using Duncan's new multiple range test.

On a per plant basis, NRA per shoot ranged from 8.60 to 75.85 umoles NO_2^- produced per hour, whereas NRA per root ranged from 0.36 to 1.76 umoles NO_2^- produced per hour. Shoot NRA in W536 and W627C was significantly higher than in 12 other genotypes. On the average, total root NRA in these genotypes was about 3% of the shoot NRA. W536 and W627C were significantly higher in total plant NRA than were 12 other genotypes, and W117, W182BN, and A632 were significantly lower than were seven other genotypes.

Genotypes with high shoot NRA also had high root NRA, as shown in Figs. 1 and 2. Correlation coefficients for shoot versus root NRA were 0.786 and 0.787, respectively, for NRA based on a per gram fresh weight basis and for a per plant part basis.

Nitrogenous Fractions

Total N, nitrate, and reduced N differed significantly among the 16 genotypes (Table 3). In shoots, TN ranged from 18.87 to 47.96 mg N, whereas root TN ranged from 2.75 to 5.54 mg N. Total N per plant ranged from 23.07 to 53.37 mg N. Shoot TN in W739A and W627C was significantly higher than in 10 other genotypes. Root TN in W739A and W536A was significantly higher than in four other genotypes. Plant TN for W739A was significantly higher than for 10 other genotypes. Because nitrate was the sole source of N provided to these seedlings, and because TN measurements include nitrate, TN should be indicative of the net influx of nitrate-N into the plants. Therefore, genotypic differences in uptake of N were observed in this study.

Nitrate in shoots and roots ranged from 3.18 to 11.98 mg N and from 0.66 to 1.86 mg N, respectively (Table 3). Plant nitrate ranged from 3.94 to 13.33 mg N. Shoot nitrate in W627C

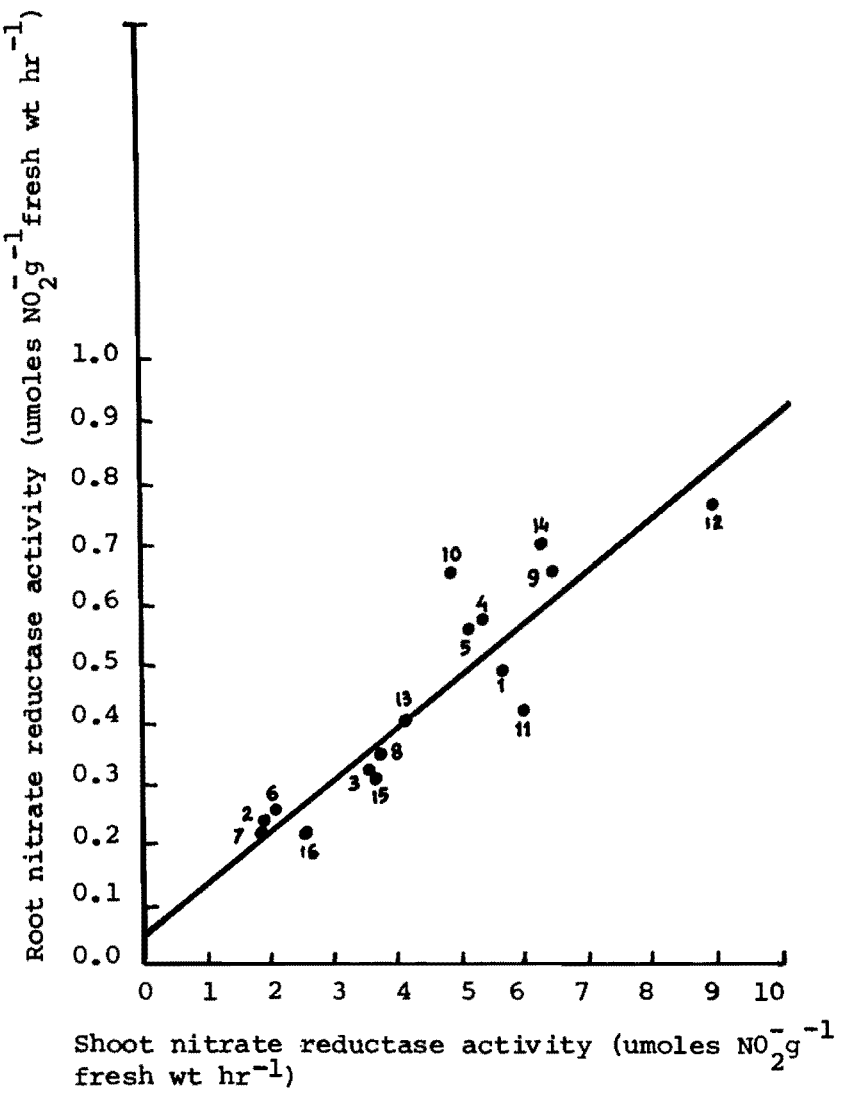

Fig. 1. Relationship between Root Nitrate Reductase Activity (NRA) and Shoot NRA per Gram Fresh Weight in 16 Corn Inbreds.

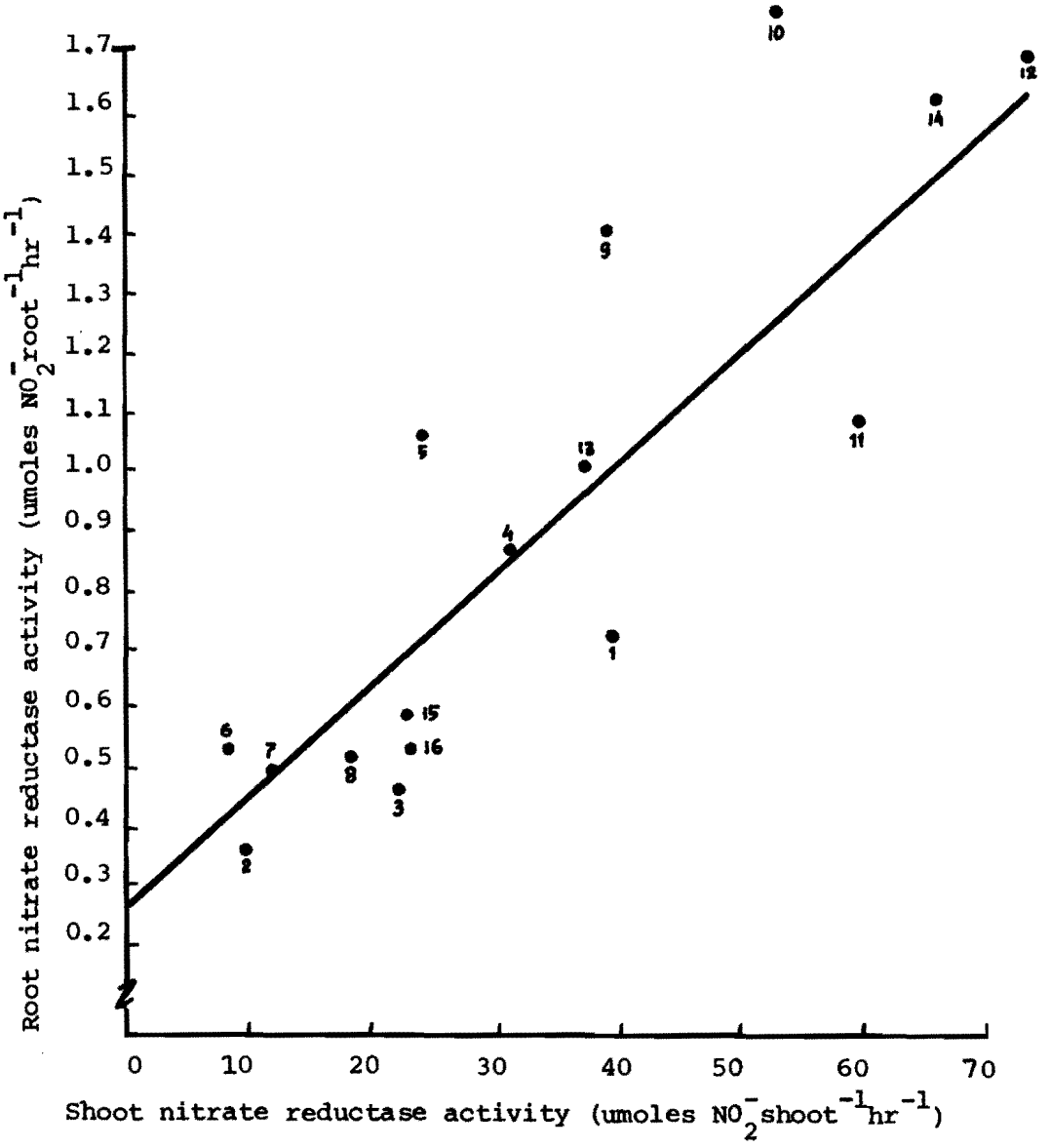

Fig. 2. Relationship Between Root Nitrate Reductase Activity (NRA) and Shoot NRA per Plant Part in 16 Corn Inbreds.

Table 3. Partitioning of Total N (TN), Nitrate and Reduced N (RN) Between Shoots and Roots of 16 Corn Inbreds

	Genotype	Total N			Nitrate^{-N}			Reduced N		
		Shoot	Root	Plant	Shoot	Root	Plant	Shoot	Root	Plant
		mg plant^{-1}								
1.	A619	32.53bc*	2.75b	35.28cdef	4.26de	0.50d	4.76e	28.27bcdef	2.52c	30.51bcdef
2.	W117	20.12d	2.95b	23.07f	3.26e	0.68d	3.94e	16.86gh	2.27c	19.13g
3.	W182E	28.27cd	3.11b	31.38ef	4.96de	0.68d	5.64de	23.32defg	2.43bc	25.74def
4.	W182B	27.65cd	3.62ab	31.27ef	6.00cde	0.8/bcd	6.87cde	21.65efgh	2.75bc	24.40efg
5.	W153R	28.77cd	4.54ab	33.31cdef	3.55e	0.68d	4.23e	25.22cdefg	3.86ab	29.08cdefg
6.	W182BN	18.87d	4.44ab	23.31ef	3.18e	0.96bcd	4.14e	15.69h	3.48abc	19.17g
7.	A632	23.01cd	3.51ab	26.52ef	4.72de	0.77cd	5.49de	18.29gh	2.74bc	21.03fg
8.	C123	26.76cd	3.17b	29.93ef	4.57de	0.77cd	5.34de	22.19efgh	2.40bc	24.59efg
9.	Oh43	33.60bc	3.62ab	37.22bcde	3.50e	0.66d	4.16e	30.10abcde	2.96abc	33.07abcde
10.	W739A	47.96a	5.41a	53 37a	10.10ab	1.86a	11.96ab	37.87a	3.55abc	41.42a
11.	W59M	41.88ab	4.14ab	45.32abcd	7.52bcd	1.33abcd	8.85bcd	33.66abc	2.81abc	36.47abc
12.	W536	43.90ab	5.54a	49.44ab	8.48bc	1.34abcd	9.82abc	35.42ab	4.20a	39.62ab
13.	W729D	41.91ab	4.35ab	46.26abcd	9.94ab	1.63ab	11.57ab	31.57abcd	2.72bc	34.69abcd
14.	W627C	46.58a	4.03ab	50.61ab	11.98a	1.35abcd	13.33a	34.60ab	2.67bc	37.27abc
15.	W438	28.62cd	4.47ab	33.09def	8.77abc	1.34abcd	10.11abc	19.85fgh	3.13abc	22.98efg
16.	W64A	42.14ab	4.68ab	46.82abc	10.27ab	1.57abc	11.84ab	31.87abcd	3.11abc	34.99abcd

*Values in a column followed by the same letter are not significantly different at the 5% level using Duncan's new multiple range test.

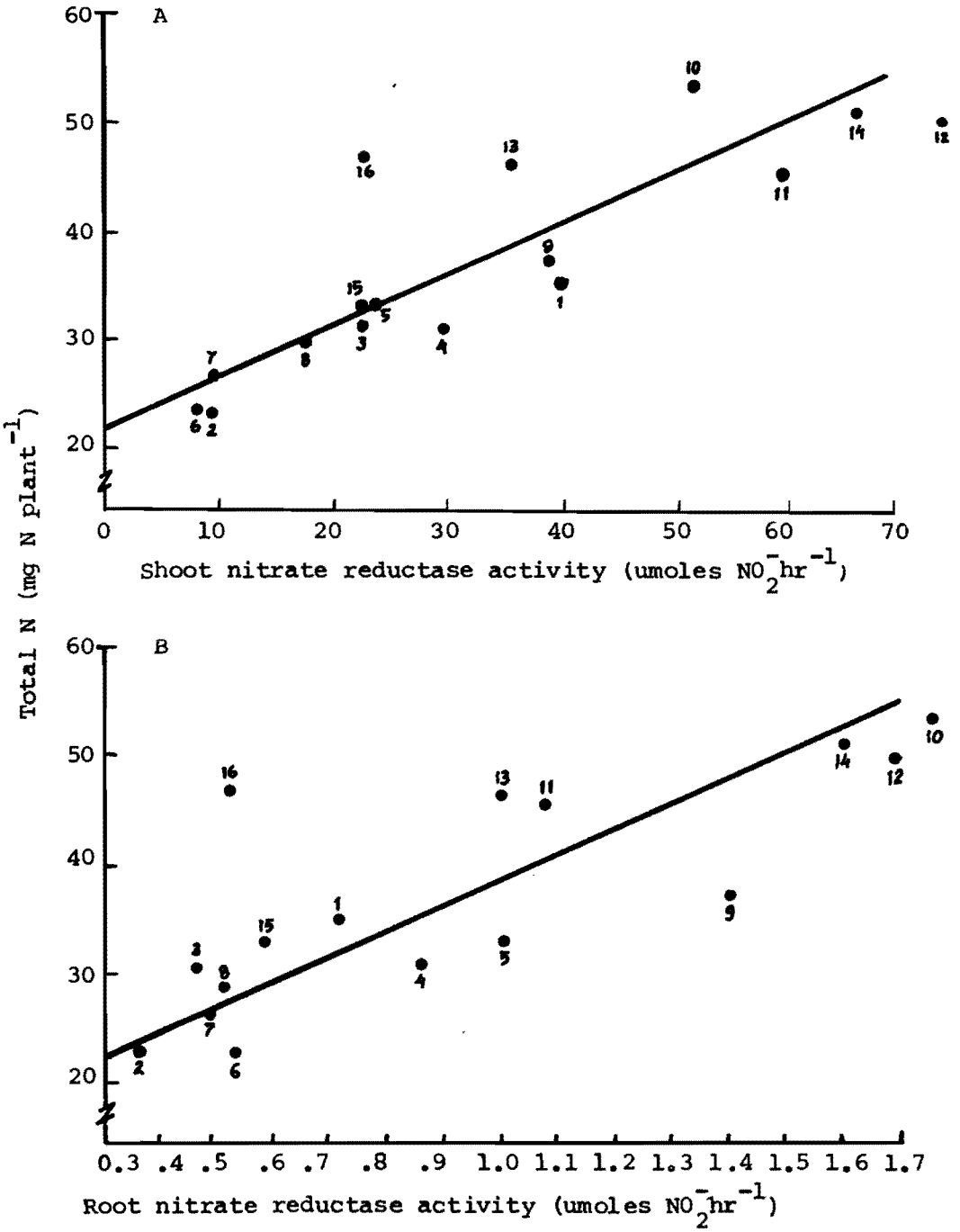

Fig 3. Relationship Between Total N and Shoot Nitrate Reductase Activity (NRA) (A) and Root NRA (B) in 16 Corn Inbreds.

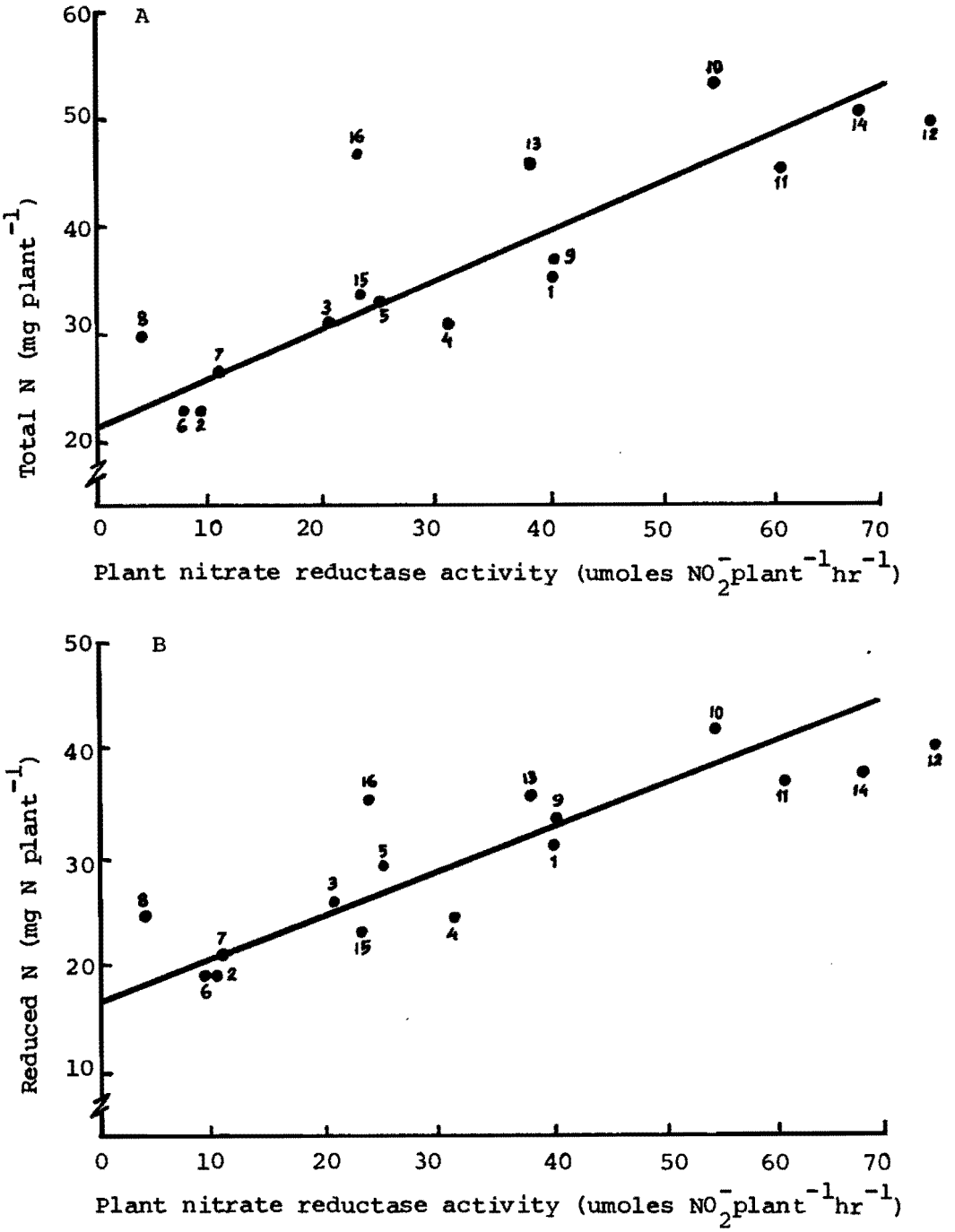

Fig. 4. Relationship Between Plant Nitrate Reductase Activity (NRA) and Total N (A) and Reduced N (B) in 16 Corn Inbreds

was significantly higher than in 11 other genotypes. Root nitrate in W739A was significantly higher than in nine other genotypes. W627C, W739A, W729D, W64A, W438, and W536 were significantly higher in plant nitrate than were A619, W117, W182E, W153R, W182BN, A632, C123, and Oh43.

In shoots, RN ranged from 15.69 to 37.87 mg N, whereas root RN ranged from 2.25 to 4.20 mg N. Plant RN ranged from 19.13 to 41.42 mg N. Shoot RN in W739A was significantly higher than in nine other genotypes. Root RN in W536 was significantly higher than in eight other genotypes. W739A, W59M, W536, W729D, W627C, W64A, and Oh43 were significantly higher in plant RN than were W117, W182BN, and A632.

Genotypes with high shoot and root NRA also had high TN as shown in Fig. 3. The correlation coefficient for shoot NRA versus plant TN was 0.772 whereas the correlation coefficient for root NRA versus plant TN was 0.727.

On a per plant basis, genotypes with high NRA also had high TN and RN (Fig. 4). The correlation coefficients for plant NRA versus TN and RN were 0.775 and 0.781, respectively.

DISCUSSION

The observed genotypic differences in root size (Table 1) appeared to be related to the observed differences in accumulated N per plant (Table 3). However, total N accumulated per gram fresh weight of roots varied from 11.1 to 25.0 mg N per gram of roots, and only 10 of the 16 genotypes had a ratio between 16.9 and 20.4. Thus, factors other than root size affect nitrate absorption. Earlier reports (2, 7, 10, 18, 19) attributed observed genotypic differences in ion uptake to variations in root size and morphology as well as to differing capacities for ion

uptake. Chevalier and Schrader (7) compared nitrate uptake by four of the inbreds included in this study to uptake by their F_1 progenies. Nitrate absorption and accumulation were highest for W64A and lowest for A632, and Wl82E and Oh43 were intermediate (7). In the present study, only three inbreds accumulated more nitrogen than did W64A and only two accumulated less than did A632. Only two inbreds accumulated more nitrate-N than did W64A. A632 was among a group of inbreds that accumulated significantly less nitrate-N than did W64A. Hence the results of these two studies are comparable even though plants were 12 weeks old in the former study (7) as compared to 2 weeks old in the present study. Both studies indicate that selection for genotypic variation in maize inbreds for uptake or accumulation of nitrate is possible.

The positive correlation between root and shoot NRA was not anticipated. Pate (16) reported that species with high root NRA transport little nitrate to the shoots whereas species with low NRA in roots transport a large percentage of their N to the shoot in the form of nitrate. The capacity for nitrate reduction in root tissues is fairly readily saturated and as the external supply increases, the proportion of nitrate relative to reduced N in xylem exudates rises (15, 27). Therefore, under the same external nitrate supply, genotypic variations in root NRA should result in different proportions of nitrate relative to reduced N in the xylem. On the basis of these reports, one might predict an inverse relationship between root and shoot NRA in these inbreds. However, a positive relationship was observed between root and shoot NRA (Figs. 1 and 2) suggesting some form of integrated control over uptake and translocation of nitrate throughout the seedlings and over the maintenance of an inducing pool of nitrate in each region, as described by Wallace (26).

Aslam and Oaks (1) observed that activities of NADH-NR, $FMNH_2$-NR, and nitrate-induced NADH cytochrome c reductase in corn leaves and roots were induced in parallel in both tissues when nitrate was supplied. Our data also suggest that root and shoot NR are both induced in parallel when high levels of nitrate are provided to maize seedlings. In contrast, Reed and Hageman (20) recently reported that NRA in four maize hybrids was not correlated with accumulation of nitrate or reduced N. Uptake and flux of nitrate were not numerically related to accumulation of reduced N, nor was nitrate flux associated with differences among the four genotypes in leaf NRA.

Butz and Jackson (4) proposed that NR serves both a transport and reduction function. According to their model, an NR tetramer is oriented such that one monomer is exposed to the outside of the plasmalemma while the other three subunits are exposed to the cytoplasmic side. A mechanism was suggested for this model whereby the transport and reduction of one nitrate ion is accompanied by the transport of two additional nitrate ions. According to their model, one-third of the nitrate would be reduced in roots and two-thirds would be available for transport to other plant parts. Although we observed a high correlation ($r = 0.727^{**}$) between root NRA and total N accumulated (Fig. 3), root NRA was very low relative to shoot NRA (Table 2). Casein was added to the extraction media to stabilize NRA (23), but root NRA averaged only about 3% of the total *in vitro* NRA per plant in the 16 genotypes. In younger maize seedlings, Wallace (26) reported less than 20% of the total NRA in the root, and Robin et al. (21) observed that NRA in roots was about 20% of the NRA in corn seedlings. Therefore, three investigators have reported root NRA somewhat lower than the 33% predicted by the model of Butz and Jackson (4). It is acknowledged that these

in vitro studies may not accurately reflect the sites of nitrate reduction *in situ* (13). Although the proposal that NR is involved in nitrate uptake cannot be ruled out at this time, these data on *in vitro* NRA do not support the concept that one-third of the nitrate is reduced as it is absorbed by the roots.

Although highly significant correlations between root NRA, shoot NRA, and TN were observed (Fig. 3), root NRA in 16 genotypes was about 3% of the shoot NRA which suggests that shoot NRA plays a much greater role than does root NRA in N assimilation in corn seedlings. The observed positive correlations between plant NRA and RN of the 16 maize inbreds used in this study (Fig. 4) support the concept that *in vitro* assays of NRA may be used to estimate the potential of maize seedlings grown on high nitrate to convert nitrate-N to RN as suggested earlier (8, 9).

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