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Partitioning into Maize Grain N Fractions of N Absorbed Through the Roots Before and After Pollination

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The abundance of N in various fractions in maize (*Zea mays* L.) grain was compared in two genotypes, a normal hybrid (Pioneer 3369A) and its *opaque*-2 (*o*-2) counterpart (L3369). Plants were grown in the greenhouse in sand cultures irrigated daily with nutrient solutions containing 3.75 mM N as NO_3^- ion. At anthesis the plant cultures were divided into sets, one set continuing to be irrigated with the same solution, but with ^{15}N added, and the other set irrigated with a solution of the same composition except that the N was omitted. The plants were sampled at 12, 24 and 36 days after pollination. Post-anthesis N nutrition affected the composition of the vegetative growth but had no effect during the sampling period on yields or amounts of N in the grain. At 12 days post-pollination there were no differences between the two genotypes in N partitioning, with about 50% of the N present as nonprotein N (NPN). At 24 and 36 days over 75% more N was present as zein in the normal genotype as in the *o*-2, while the latter contained a much higher proportion of N as NPN. The albumin-globulin and glutelin fractions were slightly greater in the *o*-2. The N absorbed after pollination was partitioned among the grain protein fractions similarly to N absorbed prior to pollination. The nature of the NPN fraction needs to be considered in evaluating effects on crop quality of an elevated N supply.

Keywords: Maize grain; N nutrition; ^{15}N; N fractionation; nonprotein N; zein.

1. Introduction

Understanding the manner in which absorbed N is partitioned in crops during grain fill is important in improving the efficiency of N use in cereals through fertiliser management practices and plant breeding. Especially in cereals which contain prolamine proteins (proteins soluble in aqueous alcohol solutions), such as the zein fraction in maize, N partitioning in the grain also provides clues to the influences of nutrition on grain quality. In maize genotypes generally, zein is a dominant N sink and becomes increasingly abundant in the grain under conditions of high soil N levels.[1–4] This protein fraction contains very low amounts of the essential amino acids, lysine and tryptophan. The preferential accumulation of grain N in zein has been shown to be decreased by mutation at the *opaque*-2 (*o*-2) locus.[5] Yields of *o*-2 are generally lower, and have been attributed to metabolic influences resulting from the accumulation of amino acids and their catabolic products.[5] The effectiveness of zein as an N sink includes the dynamic aspects, i.e. the duration of the period of synthesis of this protein fraction during grain fill.[6] Results from other recent studies indicate that recovery of N in maize grain is maximised when applied to the crop late in the vegetative period of growth.[7] The objective of the present study was to determine how N absorbed by maize roots was partitioned into various grain N fractions at three sampling times, in order to gain a better understanding of the dynamic aspects of grain N accumulation. Through use of ^{15}N, it was possible to identify the amounts of N which were absorbed through

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the roots during the post-pollination period, and accumulated in various N fractions in the grain. Two genotypes, one containing the *o*-2 gene, and the other its normal hybrid counterpart were compared.

2. Experimental

2.1. Growth of seedlings and culture techniques

Seeds of a commercial maize hybrid, Pioneer 3369A (normal) and Pioneer L3369, its *o*-2 counterpart, were germinated by placing on paper towels in contact with 0.2 mM $CaSO_4$. Because more rapid germination of 3369A had been previously observed,[8] seeds of L3369 were placed in the germination chambers 2 days before those of 3369A. After selection for uniformity, the seedlings were transplanted, two per genotype, in each of 24 pots containing 17.2 kg of washed sand. Fifteen days after emergence the plants were thinned to one normal and one *o*-2 per pot. During the time from transplanting the seedlings to pollination, all plants were watered with nutrient solution containing 3.75 mM N as NO_3^- ion, but otherwise similar to a modified Hoagland solution.[9] The plants were manually pollinated as previously described.[8] Open pollination was prevented by covering silks and tassels with bags. Two N treatments were begun at pollination. From time of pollination until final harvest 12 pots were irrigated with the same modified Hoagland nutrient solution but containing no N. The other 12 pots received nutrient solution identical to that provided prior to pollination, but with ^{15}N (9.20 atom % excess) added.

2.2. Harvesting, sampling and analysis

Plants from four pots were harvested at 12, 24 and 36 days after pollination, frozen, and the samples stored in a freezer. The grain was shelled in the cold after dipping the ears into liquid N_2. The samples were lyophilised, ground and dried at 70°C to constant weight. Total N was determined by a Kjeldahl method.[10] Lipids were removed from samples of the ground grain with petroleum ether (ligroine 30–60°C), and the N fractions extracted using a modification of the Landry and Moreaux[11] procedure, incorporating some changes suggested by Sodek and Wilson.[12] After defatting, the samples were extracted with 0.5 M NaCl to remove both the globulin and albumin fractions. This extract was treated with 10% TCA, and the soluble portion was separated and analysed to determine nonprotein N (NPN). Total N of each fraction was determined by the Kjeldahl method. The data were subjected to statistical analyses as indicated on the tables and figures.

3. Results and discussion

There were no statistically significant differences between the two genotypes in grain yield or N concentration. The mean grain yields were similar whether or not N was included in the solution used in the post-pollination period for the daily irrigation of the cultures. As noted in a previous publication,[13] amounts of N in the stalks and leaves were greater in the plants which continued to receive N until harvested, but this difference in N nutrition did not have any significant effect on N accumulation in the grain (Table 1). This lack of response in grain N to the continuing supply of N was also noted in the studies of Friedrich and Schrader.[14] Storage of N in the vegetative parts of the plants during the growth period were sufficient to meet grain filling needs. The amounts of total N accumulated are comparable to those reported by Below *et al.*[15] for four hybrids grown in field plots. For the whole ear, during the 7 to 42-day grain-fill period, they obtained values ranging from about 1800 to 2400 mg plant^{-1}. The quantities of N found in the grain in these studies (Table 1) were within this range. Most of the N in the ear would be present in the grain, relatively little was found in the cob and husks.[13]

Changes during the 36-day post-pollination period in the percentages of extractable N in the various grain fractions are shown in Table 2. Since there were no significant effects of N treatment on total N contents or fractions in the grain, the values shown are means from both the 0 and 3.75 mM N post-pollination treatments. As expected, there was a large difference between

Table 1. Amounts of total N in stems, lower leaves, upper leaves and grain of maize plants at 12, 24 and 36 days after pollination; data for Pioneer GH3369A (normal) and Pioneer L3369 (*opaque*-2) combined

Plant part	Days after pollination	N treatment (mM) 0 (mg plant^{-1})	3.75 (mg plant^{-1})	Significance[a]
Stalks	12	1038	1368	**
	24	566	1218	***
	36	502	1266	***
Lower leaves	12	654	758	**
	24	500	680	**
	36	237	556	***
Upper leaves	12	527	538	NS
	24	420	516	**
	36	318	415	**
Grain	12	281	316	NS
	24	1150	1240	NS
	36	2058	2091	NS

[a]Confidence levels for differences between means in same row are NS: not significant at $P<0.05$; **: $P<0.01$; and ***: $P<0.001$.

Table 2. Partitioning of extractable grain N into various N fractions of two maize genotypes at three sampling dates, expressed as percentages of total grain N, with standard deviation of means

Days after pollination	NPN (%)	Albumin globulin (%)	Zein (%)	Glutelin (%)
Normal genotype				
12	50±5.5	14±1.9	4±2.0	18±2.0
24	15±1.2	11±2.8	40±1.3	16±1.7
36	11±0.9	12±0.8	47±2.7	16±1.6
o-2 genotype				
12	55±4.0	14±3.3	2±0.2	17±1.4
24	27±3.4	15±4.1	22±1.3	20±1.8
36	22±2.2	16±0.9	26±2.1	21±1.4

the two genotypes in percentages of N in the zein fraction,[3] although the amounts were very low in both at the 12-day sampling. There were also much larger percentages of NPN in the *o*-2 grain than in the normal grain at the 24-day and 36-day samplings. At these sampling dates the *o*-2 grain also contained somewhat larger percentages of the albumin-globulin and glutelin fractions. Had the grain been more mature at the final harvest, it is likely that the percentages of N in the NPN fraction might have decreased, and the zein N fraction increased slightly.

Very similar values were found for the percentages of N in the various fractions, whether the calculations were based on the grain N taken up after pollination (Table 3) or on the total amount in the grain (Table 2). This indicates that the mechanisms involved in partitioning function similarly as regards synthesis of grain protein from N mobilised from that previously absorbed and stored in the vegetative tissues, or from N absorbed through the roots during grain fill.

Of the total N found in the grain in the various fractions on the three sampling dates, about one-sixth was accounted for by that incorporated during grain filling (exogenous N) into NPN of both genotypes at 12 days (Table 4). At the two subsequent samplings, this fraction in the *o*-2 contained a larger portion of the exogenous N than its normal counterpart. Only very small amounts of exogenous N had been used in zein biosynthesis at 12 days, but at both the 24- and 36-day samplings only about half as much of the exogenous N was found in the *o*-2 as in its normal counterpart.

Table 3. Partitioning of extractable grain N into various N fractions of two maize genotypes at three sampling dates, expressed as percentages of grain N absorbed during post-pollination (exogenous N), with standard deviation of means

Days after pollination	NPN (%)	Albumin globulin (%)	Zein (%)	Glutelin (%)
Normal genotype				
12	52.4±8.6	11.3±3.7	3.5±2.3	17.4±4.1
24	12.6±3.2	10.5±1.7	43.6±1.3	14.6±2.6
36	9.7±1.6	12.4±1.0	51.1±3.0	13.7±0.8
o-2 genotype				
12	60.0±9.2	16.8±5.5	1.4±0.5	15.1±1.2
24	24.8±6.0	13.3±4.5	21.9±1.6	17.5±1.2
36	25.8±3.3	18.0±1.6	25.1±4.2	20.1±1.1

Table 4. Nitrogen taken up by plants during the post-pollination period and incorporated into various fractions, expressed as percentages of the total grain N, with standard deviation of means

Days after pollination	NPN (%)	Albumin globulin (%)	Zein (%)	Glutelin (%)
Normal genotype				
12	16.0±3.3	3.7±1.7	1.1±0.8	5.7±1.9
24	5.0±1.7	4.2±0.9	17.2±2.4	5.7±0.5
36	3.7±0.5	4.7±0.1	19.5±1.9	5.2±0.6
o-2 genotype				
12	17.1±2.5	4.9±1.9	0.38±0.1	4.3±0.7
24	9.0±3.1	4.7±1.6	7.8 ±0.5	6.2±0.6
36	9.3±1.7	6.5±0.6	9.0 ±1.2	7.2±0.1

The marked effect of the presence of the *o*-2 gene on the occurrence of NPN was also found by Sodek and Wilson.[12] From analysis of the TCA-soluble portion of a water extract of endosperm they found that 4.4 and 19.7% of the total N in the endosperm of mature grain from an R802 genotype and its *o*-2 counterpart, respectively, was present as free amino acids. In their study, aspartic and glutamic acids, and asparagine accounted for well over half of the N in the free amino acid fraction of both genotypes. Proline and glutamine were also present in large amounts but differed in the two genotypes. The concentrations (μmol basis) of these two amino acids were 19.6 and 4.9, respectively, in the normal genotype, and 8.6 and 15.9, respectively, in the *o*-2 genotype.

Because of the preponderance of nonessential amino acids in the NPN fraction, quality effects of abundant N during crop growth as measured by Kjeldahl analysis may be misleading. This response should be taken into account in evaluating grain composition effects from late application of fertiliser N.[16]

As another view of the manner in which the N absorbed during grain-fill is partitioned, and as a basis for considering physiological implications, the percentages of the N found in the various fractions which were taken up by the plants during the period between pollination and each of the three sampling dates are shown in Figure 1. Less contrast between the two genotypes is shown in the percentages of N in the various fractions when expressed on the basis of exogenous N rather than in terms of the total N in the grain as in Table 4. In both genotypes, between 30 and 40% of the NPN found at each sampling time was taken up from the nutrient solution during grain fill. For all of the protein fractions the contribution from the exogenous source increases with time up to 24 days. The magnitude of the changes in the percentage contributed was greatest in the case of the zein fraction for both genotypes. The percentage values were

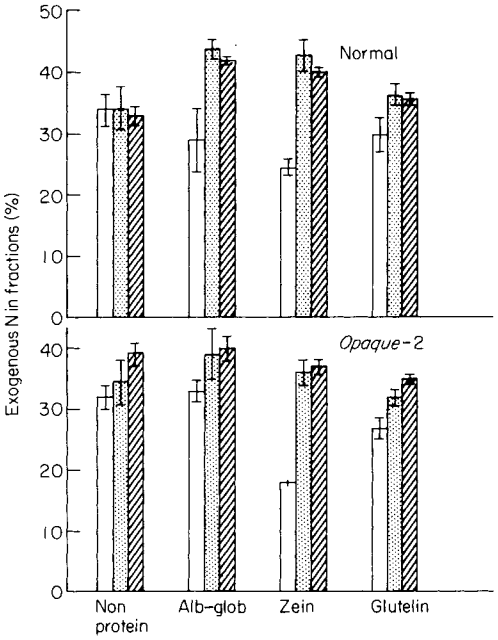

Figure 1. Percentages of the N in each of four grain N fractions attributed to post-pollination N uptake at three sampling times: □, 12; □5, 24; □, 36 days after pollination. Interval bars indicate standard deviations of means.

consistently lower for the *o*-2 genotype. While, as noted previously, the grain at the 36-day harvest was not fully matured, it does not appear that the percentages in the various fractions would undergo much further change.

With other genotypes and under other conditions of growth, responses varying in degree from those observed in these studies are likely. Features such as ear number, kernel size and number, and rate of maturation are important in determining the manner in which grain serves as an N sink.[6,17–20]

4. Conclusions

At least under conditions of growth such as used in these studies, sufficient N can be stored in maize plants before pollination to provide the needs of the grain up to 36 days post-pollination. Zein was found to be the dominant N sink in the grain of a normal maize hybrid but in its *o*-2 counterpart there was a more nearly equal partitioning between the zein, albumin-globulin, glutelin and NPN fractions. The N absorbed through the roots during the post-pollination period of growth (exogenous N) was partitioned in the grain N fractions similarly to that absorbed earlier. For the NPN fraction, throughout the post-pollination period of growth about one-third of the total amount present was accounted for by post-pollination N absorption for the normal genotype, but was slightly greater for the *o*-2. For all three of the protein fractions, the proportions of N from exogenous sources increased between 12 and 24 days post-pollination but did not change further in the subsequent 12 days.

A better understanding of the NPN fraction, in its relationship to the metabolic events associated with grain filling and to grain quality is needed to help define the influence of nutrition on N partitioning in grain proteins.

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