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A Proposed Role of Zein and Glutelin as N Sinks in Maize[1]

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ABSTRACT

***Zea mays* grown with high levels of N fertilizer transports more sucrose into kernels than with low N. Sucrose translocation was greatest in genotypes with the highest capacity to deposit nitrogenous compounds as zein and glutelin in the kernel. These two proteins combined contain about 80% of the total N in the kernel and about 60% of the total N in the plant at maturity. They appear to serve as a functional N sink for the deposition of nitrogenous compounds. As the N sink capacity increases with additional available N fertilizer, more sucrose is transported into the kernel, resulting in increased kernel weight and grain yield. Zein functions as a more dynamic N sink than glutelin because the synthesis of zein is readily manipulated by N fertilization and genetic means. Increases in N deposition in the normal endosperm induced by N fertilizer are confined primarily to zein. Early termination of zein accumulation in the *opaque-2* mutant results in a reduction of sucrose movement into kernels. By using plants heterozygous for normal and *opaque-2* in these studies, interplant variability was eliminated and the hypothesis relating the kernel N sink capacity to productivity was strengthened.**

Our previous studies (21) have suggested that zein and glutelin serve as a N sink in maize (*Zea mays* L.) kernels to regulate the movement of photosynthates into kernels. Although both ammonium and nitrate ions can be taken up by maize roots, the assimilation of ammonia and its subsequent organic N interconversions require readily available organic acids, *e.g.* α-ketoglutaric acid (12), which are derived from sucrose. In response to high concentrations of ammonia, a greater amount of sucrose is translocated from leaves to provide energy and essential carbon skeletons for ammonia assimilation and organic N interconversions (25). Concurrently, the movement of sucrose to N-rich tissues may enhance CO_2 fixation in leaves (13). The increase in photosynthetic efficiency, and the translocation of nitrogenous compounds and sucrose into the kernel sink for photosynthates, should further promote the synthesis of starch and thereby increase yield. Since nitrate, unlike ammonia, may accumulate without assimilation (18), the mixture of nitrate and ammonium ions in plants may function as a "buffer" for optimizing N utilization. Ammonia enhances the immediate movement of sucrose from leaves and increases photosynthetic efficiency, whereas nitrate functions as a N reserve to be reduced later by nitrate and nitrite reductase before assimilation (4). Mineralization of soil organic N provides varying amounts of both forms of N throughout crop growth even though the bulk of fertilizer N has been previously nitrified and is in the nitrate form. By inhibiting nitrification of applied N, an even larger quantity of the more efficient ammonium form may be made available for assimilation (8). It seems that the effectiveness of the ammonium ion in enhancing sucrose movement is facilitated by the rapid assimilation of ammonia by roots and the resultant deposition of amides and amino acids in some tissue. These nitrogenous compounds are stored temporarily in the stalk and leaves during the period of vegetative growth. About 60% of the final N in maize kernels is present in the vegetative tissues at pollination; the remaining 40% is obtained from the soil subsequently (7). As the endosperm develops, the proteins in vegetative tissues are turned over, and the amino acids and newly assimilated N are transported into the kernel (2, 6). We propose that zein and glutelin function as N sinks for the deposition of these nitrogenous compounds to facilitate the movement of photosynthates into kernels. A small N sink in the kernel may result in the accumulation of free amino acids, thus generating a more negative osmotic potential to favor water but reduce solute movement into the kernel. Hence, the yield response of grain to N fertilizer is enhanced by the presence of a large N sink in the kernel.

Although all proteins may potentially function as a N sink to the extent that their synthesis reduces the accumulation of free amino acids, zein and glutelin are considered the major N sinks because they constitute approximately 80% of the kernel proteins. Unlike glutelin, however, zein is the major storage protein and its synthesis can be manipulated readily by N fertilization as well as genetic means. This suggests that zein plays the primary functional role as a N sink in the kernel. The positive correlation between zein content, kernel weight, and grain yield previously reported (21) indicates the importance of this system as a yield determinant in maize. The much reduced zein content of the *o2*[2] mutant compared to its isogenic normal counterpart (10) makes these two genotypes a model system to test further the importance of the N sink in affecting the movement of photosynthetic assimilates into the kernel. Since the genetically recessive *o2* kernels can be readily distinguished from normal kernels on the same ear, utilization of the plant heterozygous for *o2* and its normal allele permits the evaluation of this mechanism without interplant variability, interacting pleiotropic effects caused by the *o2* mutation, and environmental interactions which could otherwise confound this type of study.

MATERIALS AND METHODS

Maize Materials, Collection, and Analysis. The maize hybrid B14 × B37, the homozygous *o2* mutant (B14*o2* × B37*o2*), and the heterozygote (B14*o2* × B37) were grown at the Purdue Agronomy Farm in 1978 on randomized, replicated (four times) field plots (4 × 45 m) spring-treated prior to planting with different rates (0, 67, 132, 201, 168, and 447 kg N/ha) of anhydrous ammonia. The zero N plot contained about 15 kg N/ha residual N plus mineralizable soil N. The normal and heterozygous plants, but not *o2* plants, were detasseled, permitting homozygous normal and *o2* plants to be open-pollinated with *o2* pollen. Kernels from these two genotypes were hand-harvested at maturity to study the effect

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[2] Abbreviation: *o2*: *opaque-2*.

of N fertilizer rates on the accumulation of various protein fractions in their endosperms. The rate of zein accumulation as affected by N rates during kernel development, and its effect on the movement of photosynthetic assimilates into the kernel of *o2* and its normal counterpart were investigated by using the heterozygote to minimize environmental effects and plant variation. The heterozygous plants were hand-pollinated with *o2* pollen to establish an accurate date of pollination. Pollination of the heterozygous plants with *o2* pollen resulted in an equal segregation for normal and *o2* kernels. One day after hand pollination, the ear was exposed to open pollination by *o2* pollen to ensure complete filling and uniform competition for N. Since the visually opaque, floury texture of the *o2* endosperm can be distinguished as early as 25 days postpollination, ear samples of the heterozygous plants were harvested at 26, 35, 39, and 45 days postpollination and at maturity. At each developmental stage, while ears were frozen in liquid N_2 immediately after harvest, kernels were cut from the frozen ears and bulked (at least four ears for each developing stage), and *o2* and normal kernels were separated and stored at −20 C.

After embryos were excised from frozen kernels, endosperms were lyophilized to a constant weight, ground in a Waring Blendor, powdered in a miniature ball-mill (Wig-L-Bug, Crescent Dental Mfg. Co., Chicago, Ill.) for 5 min, and then defatted for 48 h with *n*-hexane in a Soxhlet apparatus. The defatted sample provided the starting material for the fractionation of albumin, globulin, zein, and glutelin (22). Each 100-mg sample was shaken with 1 ml water for 15 min at 4 C followed by centrifugation at 18,800*g* for 5 min. This was repeated twice and the supernatants from these three washes were combined and regarded as the water-soluble protein, albumin. The remaining residue, following water extraction, was treated twice with 1 ml 5% NaCl at 4 C. The combined NaCl-soluble fraction was referred to as globulin. The residue remaining from the NaCl treatment was suspended in 1 ml of water to lower the salt concentration and centrifuged. The supernatant was discarded and the residue was shaken for 30 min at 60 C with 0.5 ml 95% ethanol (final ethanol concentration, about 70%) containing 1 mM 2-mercaptoethanol. After centrifuging at 18,800*g* for 5 min, the pellet was shaken again with 0.5 ml of 70% ethanol containing 1 mM 2-mercaptoethanol for 60 min at 60 C and centrifuged at 18,800*g* for 5 min to recover the supernatant. The ethanol extracts were combined and regarded as zein. Glutelin content was determined by subtracting albumin, globulin, and zein from total protein, measured by a micro-Kjeldahl method (3). The presence of 1 mM 2-mercaptoethanol in hot alcohol (70% ethanol, 70% isopropanol, or 55% isopropanol) removes over 95% of the zein. Only trace amounts of zein proteins were detectable in the residue remaining from the alcohol plus 2-mercaptoethanol extraction (24). Endosperms were analyzed because zein and glutelin are localized primarily in the endosperm (22) and they may account for about 80% of the total endosperm N. Results are expressed on the basis of the biological unit, *i.e.* the individual endosperm.

N distribution in various parts of the maize plant was analyzed at maturity by harvesting three plants from each fertilizer treatment, separating them into leaves, stalks, husks and shanks, cobs, and grains, and then determining the dry weight and N content of each tissue.

Movement of ^{14}C-labeled Sugars into the Kernel. $^{14}CO_2$ was supplied to heterozygous plants at 26, 39, and 45 days postpollination. Two plants at each stage of kernel development in each of two fertilizer treatments (0 and 201 kg N/ha) were used. Each plant received $^{14}CO_2$ released from 0.4 mg of $Ba^{14}CO_3$ containing 100 μCi for 60 min (21). Preliminary studies indicated that $^{14}CO_2$ incorporation for the normal kernel would be about 40,000 cpm/kernel for this quantity of $Ba^{14}CO_3$. Twenty kernels each of normal and *o2* were harvested from each segregating ear 6 h after treatment. Incorporation of total radioactivity into kernels was surveyed by crushing individual kernels in a scintillation counting vial and then incubating overnight at 37 C in Omnifluor (New England Nuclear) containing 3% (v/v) Protosol (New England Nuclear) before counting in a Beckman LS-100C scintillation counter. Untreated normal and *o2* kernels from the same stage of development were processed in a similar manner, and a known quantity of ^{14}C-labeled sucrose was added to the vial to determine the percentage of quenching.

Determination of Sucrose and Amino Acid Content. Sucrose and amino acids were extracted from the developing endosperms by homogenizing in 70% ethanol followed by centrifugation at 18,800*g* for 15 min. The extraction procedure was repeated twice (20). Two volumes of water were added to the combined supernatant fractions from these three extractions to precipitate ethanol-soluble proteins. The protein-free supernatant fraction then was used for the determination of free amino acids (11) and, after isomerizing the endosperm fructose, for sucrose (15). For the quantitative determination of amino acids, leucine was used as a standard.

RESULTS

Distribution of Nitrogen in Homozygous Normal Plant Tissues at Maturity. When homozygous normal maize plants were grown with 201 kg N/ha, the dry weight at maturity of leaves, stalk, husks and shank, cob, and grains accounted for 12, 22, 9, 6, and 51%, respectively, of the above-ground dry weight of the plants (Table I). About 70% of the total N was found in the grains. Grain proteins separated according to solubilities into albumin (water-soluble), globulin (salt-soluble), zein (ethanol-soluble), and glutelin (alkali-soluble) constituted about 10, 9, 41, and 40% of the total protein, respectively. Thus, zein and glutelin combined contained about 80% of the total N in the whole kernel or about 60% of the total N in the plant at maturity.

Accumulation of Protein Fractions in the Mature Endosperm of Homozygous Normal and *o2* as Affected by Rates of N Fertilizer. Homozygous normal plants were grown with different rates of N in order to study the dynamics of zein accumulation as affected by N fertilizer. Normal plants grown without N fertilizer (low N conditions), contained 0.12, 0.17, 1.07, and 1.45 mg N as albumin, globulin, zein, and glutelin in each endosperm at maturity (Fig. 1A). There was only a small increase in albumin, globulin, and glutelin per endosperm observed when the rate of N fertilizer increased up to 447 kg N/ha. With the highest rate of fertilization, these three fractions contained 0.15, 0.24, and 1.76 mg N, respectively; however, zein N increased to 2.38 mg. On the other hand, increases in N components due to N fertilizer in the homozygous *o2* mutant appeared to be proportionally distributed to all four protein fractions (Fig. 1B).

Accumulation of Zein and Non-zein Protein in the Developing Endosperm from Heterozygous Plants Segregating for Normal and *o2* Kernels under Different Rates of N. Zein accumulation in normal endosperms obtained from heterozygous plants was much lower under low N than with high N conditions (201 kg N/ha), whereas *o2* exhibited only a slight difference in zein accumulation as the rate of N fertilization increased (Table II). These results

Table I. *Distribution of N in Various Parts of Maize Plants at Maturity*

Location	Dry Weight	Nitrogen Content
	g/part	
Leaves	37	0.50
Husk and shank	18	0.09
Stalk	68	0.34
Cob	28	0.09
Grains	157	2.59

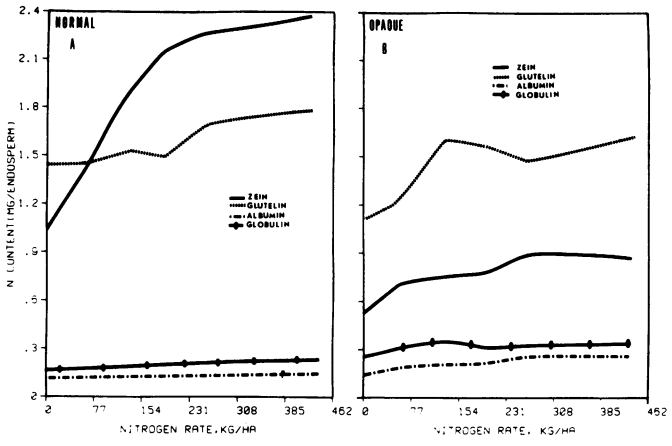

FIG. 1. Accumulation of nitrogen as albumin, globulin, zein, and glutelin from the mature endosperm of normal (A) and *o2* (B) kernels grown with different rates of N fertilizer.

agree with those obtained from homozygous kernels (Fig. 1). When grown with 201 kg N/ha, *o2* terminated zein accumulation at about 35 days postpollination, whereas zein accumulation in the normal endosperm continued for a much longer period. By maturity, *o2* endosperm contained only about 50% of the zein content of the normal genotype. Non-zein protein (albumin, globulin, and glutelin combined) continued to increase slowly in a similar manner throughout endosperm development in both the normal and *o2* mutant (Table II). These results agree with previous observations on various maize inbreds (23). Unlike high N conditions, zein accumulation in the normal endosperm with low N was only 50% higher than *o2*.

Movement of Photosynthetic Assimilates from Heterozygous Plants into Segregating Normal and *o2* Kernels during Development under Different Rates of N. Under low N conditions, there was no difference in the amount of radioactive photosynthates incorporated into *o2* and normal kernels on the same ear at 25 days postpollination. As the kernels developed, the *o2* kernels contained about 95% of the radioactivity found in normal kernels at 39 days postpollination and 88% at 45 days. However, greater differences in radioactive photosynthates were observed between *o2* and normal kernels on the same ear from plants fertilized with 201 kg N/ha. In this latter condition, *o2* kernels incorporated only 93, 86, and 54% of the radioactive photosynthates of normal kernels at 25, 39, and 45 days postpollination, respectively.

Content of Sucrose and Amino Acids in the Developing Endosperms from Heterozygous Plants Segregating for *o2* and Normal Kernels as Affected by Rates of N. Under low N conditions, both normal and *o2* contained similar levels of sucrose (mg/endosperm) throughout endosperm development (Table III). Both genotypes contained about 4.2 mg sucrose/endosperm at 26 days postpollination, and the amount declined progressively as the endosperms developed. At 45 days, both types of endosperms contained only about 25% (1 mg/endosperm) of the sucrose found at 26 days. When the heterozygous plants were grown with 201 kg N/ha, both normal and *o2* endosperms also contained about the same quantity of sucrose, 4.7 and 4.5 mg, respectively, at 26 days postpollination. However, the amounts of sucrose in the normal endosperm decreased only slightly as the endosperm developed so that, at 45 days postpollination, normal endosperms still contained about 80% (3.7 mg) of the sucrose found at 26 days postpollination. The sucrose content of *o2* endosperm decreased more rapidly during development than did that of normal endosperm, containing, at 45 days, only 2.7 mg of sucrose.

The amino acid content was lower in endosperms obtained from plants grown with low N than from those with high N. Under low N, *o2* endosperms contained amounts of amino acids only slightly higher than those of normal endosperms at comparable stages of development. With 201 kg N/ha, the amino acid content in normal endosperms declined rapidly as the endosperm developed (0.7 mg at 45 days), whereas the amino acid content in *o2* endosperms remained fairly constant, *i.e.* 2.1 mg at 45 days postpollination (Table III).

DISCUSSION

We propose that sucrose movement from leaves is facilitated by the effective assimilation of ammonium ions, whether from direct uptake or nitrate reduction, when the amides and other amino acids produced are deposited eventually in kernels as zein and glutelin. Thus, the kernels serve as a N as well as a carbohydrate sink. The data from this study, demonstrating that maize plants grown under high N conditions maintain a high level of sucrose in the endosperm throughout development and that sucrose levels decreased rapidly when N became limiting (Table III), are consistent with previous observations that assimilation of large amounts of N from soil enhances the movement of sucrose from leaves to kernels and, hence, increases yield (21). The ^{14}C data further support our hypothesis that sucrose movement into the kernel with the application of additional available N is dependent on the capacity for N deposition.

Zein and glutelin combined contain about 80% of the total N in kernels and 60% of the total N in maize plants at maturity. In serving as a N sink in the kernel, the accumulation of zein and glutelin should coincide with dry matter accumulation during endosperm development. Indeed, the weight of normal and *o2* endosperms correlates ($r = 0.85$) with the levels of endosperm N (Table II). Under N-limiting conditions, there is little difference between the zein content, non-zein protein (primarily glutelin), and weight of normal and *o2* endosperms. However, N fertilizer enhances the synthesis of zein in normal endosperm, but not in *o2*, and differences in endosperm weight between these two genotypes becomes more apparent (Table II). Difficulties in increasing the kernel weight and yield of the *o2* mutant may result from the lower N sink potential of this genotype. Increased proteins in the normal endosperm induced by N fertilization are primarily confined to zein protein (Fig. 1A) and correlate positively with endosperm weight ($r = 0.98$). In contrast, glutelin, which serves as a secondary N sink, is not as functional ($r = 0.67$) (Table II). These results agree with previous observations that zein increases preferentially in maize kernels from plants receiving high levels of N fertilizer (9, 14, 16, 17, 21) and supports previous observations that increases in kernel weight and yield are positively correlated with increases in zein content (21). Thus, zein synthesis, manipulatable by genetic means or N fertilization, is in a dynamic relationship with yield. The preferential increase in prolamin after N fertilization of barley has also been shown (1, 5, 19).

Studies of $^{14}CO_2$ feeding of maize plants heterozygous for *o2* further support the hypothesis that zein serves as an effective N sink. Since plants heterozygous for *o2* and normal alleles were used in these experiments to minimize plant variation and environmental effects, the relative amount of photosynthates moved into the two kinds of kernels on the same ear could be determined. As hypothesized, little or no difference in zein or radioactivity was observed between developing normal and *o2* kernels under low N conditions. Under high N conditions, there was also little difference in radioactivity of normal and *o2* kernels in early developmental stages prior to a large accumulation of zein. As the normal kernel develops, zein and radioactivity continue to increase. In contrast, the *o2* kernels fail to increase in zein and have a significantly lower incorporation of radioactivity than do the normal kernels.

The reduction of sucrose movement into the kernel, as affected by the termination of zein accumulation or by a reduced N sink capacity, may result from the accumulation of free amino acids or

Table II. *Endosperm Dry Weight, Zein, and Non-zein Protein Content in Developing Endosperms of Normal and o2 Kernels Obtained from Heterozygous Plants*

The plants were grown without N fertilization and 201 kg N/ha.

Time of Postpollination	No Fertilizer N						201 kg N/ha					
	Dry Weight		Zein Protein		Non-zein Protein		Dry Weight		Zein Protein		Non-zein Protein	
	Normal	*o2*	Normal	*o2*	Normal	*o2*	Normal	*o2*	Normal	*o2*	Normal	*o2*
days	*mg/endosperm*						*mg/endosperm*					
26	104	100	3.2	2.6	6.5	7.3	118	111	3.7	2.5	8.4	8.9
35							195	178	7.0	5.3	10.0	10.6
39	184	179	5.3	4.3	8.0	8.0	221	198	8.3	5.0	10.3	11.3
45	207	194	5.9	4.5	8.0	8.3	244	217	9.0	5.4	10.4	11.9
Mature	214	200	6.0	4.0	8.3	8.3	268	228	10.6	5.3	11.5	12.0

Table III. *Sucrose and Amino Acid Content in the Developing Endosperms of Normal and o2 Kernels Obtained from Heterozygous Plants*

The plants were grown without N fertilization and 201 kg N/ha.

Time of Post-pollination	No Fertilizer N				201 kg N/ha			
	Sucrose		Amino Acids		Sucrose		Amino Acids	
	Normal	*o2*	Normal	*o2*	Normal	*o2*	Normal	*o2*
Days	*mg/endosperm*				*mg/endosperm*			
26	4.2	4.1	1.3	1.4	4.7	4.5	2.7	2.8
35					3.8	3.2	1.2	3.0
39	2.2	2.1	0.9	1.4	3.8	3.3	1.0	2.8
45	1.0	0.9	0.4	0.4	3.7	2.7	0.6	2.1

their catabolic products in the kernel. The accumulation of these compounds could generate a more negative osmotic potential to favor water but reduce solute movement into the kernel. This was observed by 39 days postpollination when zein accumulation has terminated in *o2*. The *o2* kernels maintained a higher content of free amino acids (Table III) and thus higher osmotica, in contrast to the denting of normal kernels. Thus, the plump *o2* kernels could be readily differentiated from normal kernels on the same ear. This also corresponds to the period when *o2* incorporated significantly less radioactivity into the kernel, as compared to the normal. Therefore, the accumulation of zein appears to be a determining factor in increasing dry matter accumulation in the kernel and, hence, in increasing yield.

LITERATURE CITED

1. ANDERSEN AJ, B KØIE 1975 N fertilization and yield response of high lysine and normal barley. Agron J 67: 695–698
2. ARRUDA P, WJ DASILVA 1979 Amino acid composition of vascular sap of maize ear peduncle. Phytochemistry 18: 409–410
3. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1970 Official Method of Analysis, 11th ed, AOAC, Washington, D.C., p 858
4. BEEVERS L, RH HAGEMAN 1969 Nitrate reduction in higher plants. Annu Rev Plant Physiol 20: 495–522
5. BISHOP LR 1928 The composition and quantitative estimation of barley proteins. J Inst Brewing 34: 101–118
6. HANWAY JJ 1962 Corn growth and composition in relation to soil fertility. II. Uptake of N, P, and K and their distribution in different plant part during the growing season. Agron J 54: 217–222
7. HAY RE, EB EARLEY, EE DETURK 1953 Concentration and translocation of nitrogen compounds in the corn plant (*Zea mays*) during grain development. Plant Physiol 28: 606–621
8. HUBER DM, HL WARREN, DW NELSON, CY TSAI 1977 Nitrification inhibitors—New tools for food production. BioScience 27: 523–529
9. KEENEY DR 1970 Protein and amino acid composition of maize grain as influenced by variety and fertility. J Sci Food Agric 21: 182–184
10. MERTZ ET, LS BATES, OE NELSON 1964 Mutant gene that changes protein composition and increases lysine content in maize endosperm. Science 145: 279–280
11. MERTZ ET, R JAMBUNATHA, S MISRA 1975 Simple chemical and biological methods used at Purdue University to evaluate cereals for protein quality. Stat Bull No 70, p. 6, Purdue University, West Lafayette, Ind.
12. MIFLIN BJ, PJ LEA 1977 Amino acid metabolism. Annu Rev Plant Physiol 28: 299–329
13. NEALES TF, LD INCOLL 1968 The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf. A review of the hypothesis. Bot Rev 34: 107–125
14. RENDIG VV, J JIMENEZ 1978 Nitrogen nutrition as a regulator of biosynthesis of storage proteins in maize (*Zea mays* L.) grain. *In* DR Nielsen, JG MacDonald, eds, Nitrogen in the Environment. Academic Press, New York. Vol 2, p 253
15. ROE JH 1934 A colorimetric method for the determination of fructose in blood and urine. J Biol Chem 107: 15–22
16. SAUBERLICH HE, WY CHANG, WD SALMON 1953 The amino acid and protein content of corn as related to variety and nitrogen fertilization. J Nut 51: 241–250
17. SCHNEIDER E, B EARLEY, EE DETURK 1952 Nitrogen fractions of the component parts of the corn kernel as affected by selection and soil nitrogen. Agron J 44: 161–169
18. SCHRADER LE, D DOMSKA, PE JUNG, JR, LA PETERSON 1972 Uptake and assimilation of ammonium-N and nitrate-N and their influence on the growth of corn (*Zea mays* L.). Agron J 64: 690–695
19. SHEWRY PR, MA KIRKMAN, HM PRATT, BJ MIFLIN 1978 Storage protein formation in normal and high lysine barley. *In* BJ Miflin, M Zoschke eds, Carbohydrate and Protein Synthesis. European Economics Communities, Luxembourg. p 155
20. TSAI, CY, F SALAMINI, OE NELSON 1970 Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol 46: 299–306
21. TSAI CY, DM HUBER, HL WARREN 1978 Relationship of the kernel sink for N to maize productivity. Crop Sci 17: 399–404
22. TSAI CY 1980 Tissue-specific zein synthesis in maize kernel. Biochem Genet 17: 1109–1119
23. TSAI CY 1979 Early termination of zein accumulation in *opaque*-2 maize mutant. Maydica 24: 129–140
24. TSAI CY 1980 Note on the effect of reducing agent on zein preparation. Cereal Chem. In press
25. YEMM EW, AJ WILLIS 1956 The respiration of barley plants. IX. The metabolism of roots during assimilation of nitrogen. New Phytol 55: 229–252