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Effects of Vegetative Source on Zein Synthesis in Maize

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ABSTRACT : Efforts were made to study whether zein accumulation in maize endosperms was controlled by the source activity or by endosperm genotypes. Analysis of F_2 and F_3 individual kernels from segregating ears derived from crosses between maize cultivars ILP and IHP that differ in zein concentrations showed no quantitative segregation in zein. When the kernels of these cultivars were cultured *in vitro*, zein accumulation was greatly influenced by amino acid concentrations in the medium. *In vitro* labelling of developing kernels with radioactive amino acid indicated that the movement of glutamine into kernels followed a non-saturated kinetics. These results suggest that accumulation of zein in maize endosperms is determined by the source supply of nutrients rather than by the endosperm genotype.

KEY WORDS : Amino acids, Sucrose, Maize, Sink, Source, Zein, *Zea mays* L.

INTRODUCTION

Kernel is the primary sink for carbon (C) and nitrogen (N) assimilates in maize. Sucrose and amino acids provided by the maize vegetative tissues are the main carbon (C) and nitrogen (N) sources, respectively, for the synthesis of starch and storage protein in developing kernels. Zein is a major storage protein and may account for as much as 60% of total protein in the maize endosperm. Previous studies have shown that zein is important for increasing grain yield because it functions as a major N sink to regulate the movement of nutrients into kernels, thus facilitating starch accumulation in the kernel (Tsai *et al.*, 1980; Tsai, 1983).

Although zein is a major storage protein, regulation of the synthesis is still not clear. Genetic studies have shown that synthesis of zein polypeptides is controlled by several endosperm genes such as *o2* and *fl2* (Tsai *et al.*, 1978; Nelson, 1979; Tsai, 1983). However, other studies have illustrated that zein accumulation may be affected by the source supply (Tsai, 1983; Reggiani *et al.*, 1985; Lyznik and Tsai, 1989; Singletary and Below, 1989; Tsai *et al.*, 1990). In an effort to determine whether zein synthesis is regulated by the source supply or by genetic composition of the endosperm, F_1, F_2, and F_3 kernels derived from crosses between maize cultivars differing in zein concentrations were analyzed to evaluate the sink effect. Developing kernels cultured *in vitro* or incubated with reaction mixtures containing ^{14}C-sucrose and ^{3}H-glutamine of varying ratios were conducted to determine the source effect.

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MATERIALS AND METHODS

Plant Materials

Maize (*Zea mays* L.) cultivars ILP and IHP, which differ in zein concentrations, were used as parents to study the sink effect on accumulation of zein in endosperms. The zein concentrations of ILP and IHP were approximately 2% and 13%, respectively. Homozygous kernels of parents, F_1 kernels obtained from reciprocal crosses between ILP and IHP, and their F_2 kernels were planted at the Purdue Agronomy Farm. Anhydrous ammonia (270 kg N ha^{-1}) was applied prior to planting. All plants were hand-pollinated to obtain homozygous kernels, F_1 heterozygous kernels, and F_2 and F_3 segregating kernels on an ear. Also, backcrosses of F_1 plants were made to their respective parents.

In a separate experiment to determine the effect of N levels on zein accumulation in endosperms, maize hybrid B73 x LH51 was grown with different levels of N fertilizer (0, 90, 180, and 270 kg N ha^{-1}) at the Purdue University Agronomy Farm. All plants were self-pollinated and kernels were harvested at maturity.

Zein and Total Protein Determinations

At maturity, 20 kernels from randomly collected ears of the selfings or crosses were sampled and analyzed for endosperm dry weight and zein concentration on a single endosperm basis. Pericarp and embryo were removed from the kernel because zein is localized primarily in the endosperm (Tsai, 1979; Lee and Tsai, 1984). Ground endosperm samples were further powdered in a miniature ball-mill (Wig-L-Bug, Crescent Dental Mfg. Co., Chicago, IL) for 5 min and defatted for 48 h with n-hexane in a Soxhlet apparatus. Total zein was extracted with hot 70% ethanol containing 2% 2-mercaptoethanol (Tsai, 1980) and measured colorimetrically (Bradford, 1976). Total N was determined by a micro-Kjeldahl method (Assoc. Off. Anal. Chem., 1984). N concentration was multiplied by a factor of 6.25 to calculate total protein concentration.

Determination of Amino Acid Concentration

At mid-silk, leaf and stalk tissues of ILP and IHP self-pollinated plants were harvested, frozen immediately in liquid N, and stored at -20°C before amino acid extraction with 70% ethanol (Tsai *et al*., 1970) and determination with ninhydrin (Mertz *et al*., 1975).

Kernel Cultures

ILP and IHP kernels were cultured *in vitro* (Cully *et al*., 1984) to evaluate the source effect on zein accumulation. Kernels collected from field-grown plants 5 days post-pollination (DPP) were placed on agar plates containing 175 or 350 mM sucrose and 0, 22, 44, 88, or 176 mM of amino acids. Kernel cultures were incubated in the dark at 30°C for 40 days. After harvest, kernels were lyophilized before zein extraction and determination as mentioned above.

Movement of ^{14}C-sucrose and ^{3}H-glutamine into Maize Kernels

Developing kernels of B73 x LH51 grown under 270 kg N ha^{-1} were collected at 14, 21 and 28 days DPP. Movement of radioactivity into endosperm was determined according to

the method of Lyznik *et al.* (1989). After removal of the tip cap region, kernels were preincubated in 5 mM sodium phosphate buffer, pH 6.5, for 30 min.. Subsequently, five kernels were incubated in 2 ml of reaction mixture. The C/N ratios in the reaction mixtures were varied by using a pair combination of one sucrose (C) concentrations (100 mM) and three amino acid (N) concentrations (5, 20 and 80 mM). Reaction mixtures were prepared according to Cully *et al.*, (1984). To monitor the uptake of sucrose and amino acids by the endosperm, 2 μCi of radioactive ^{14}C-sucrose (350 mCi/mmole) and 1 μCi of ^{3}H-glutamine (43.8 Ci/mmole) were added to each of the reaction mixture.

After incubation for four hours, kernels were removed from the reaction mixture, washed with distilled water, and dissected to remove the pericarp and embryo. The endosperm was crushed in a scintillation vial containing 1 ml of 70% ethanol before adding 100 μl of a mixture of 70% perchloric acid and hydrogen peroxide (1:1, v/v). The mixture was incubated overnight at room temperature to decoloring the solution. After incubation, 10 ml of counting cocktail (Budget-Solve) containing 5% (V/V) protosol (New England Nuclear, USA) was added. At least 48 hours later, the radioactivity of ^{14}C and ^{3}H was determined in a liquid scintillation counter (Beckman Model LS 3801).

RESULTS AND DISCUSSION

Zein Accumulation in ILP and IHP Endosperms

As shown in Table 1, zein concentration in ILP selfed endosperms was 1.8%, while the concentration of zein in IHP was 13.0%. When reciprocal crosses were made between ILP and IHP, zein concentration in ILP x IHP endosperms and IHP x ILP endosperms were 1.7% and 14.1%, respectively, with values similar to their female parents indicating a maternal effect. These results were similar to previous studies involving crosses with various maize inbreds (Reggiani *et al.*, 1985; Singletary and Below, 1989; Tsai *et al.*, 1990). When the F_1 kernels resulting from the reciprocal crosses between ILP and IHP were planted and selfed or backcrossed (BC), zein concentration in F_2 and BC endosperms from ears of these crosses ranged from 8.3% to 10.7%, indicating an intermediate value between the ILP and IHP parents. Zein concentration in individual endosperm from each ear was similar as indicated by the small values of standard deviation.

Although there was little variation in zein concentration among the F_2 endosperms in a segregating ear, genetic segregation of zein polypeptides unique to ILP and IHP was obvious on IEF gels (data not shown). Segregation of zein polypeptides in the F_2 endosperms were expected due to the segregation of zein structural genes. The lack of quantitative segregation in zein concentration of individual endosperms within the F_2 segregating ear clearly indicates that the quantitative accumulation of zein in kernels is determined by vegetative source activity rather than by the genetic constitute of endosperms. When kernels from the F_2 "segregating" ear were subsequently planted and selfed, the total protein concentration of F_3 endosperms from each of the 14 different ears varied from 10.3% to 18.6%, a segregation toward the parents ILP and IHP; however, protein concentration of individual endosperms from each ear was similar (Table 2). Although zein concentration was not determined for the

F_3 kernels, the results obtained for total protein also showed little variation in concentration (a reflection for changes in zein concentration) among segregating endosperms. These results obtained for F_2 and F_3 support the hypothesis that zein or protein accumulation is not determine by endosperm genotypes.

Table 1. Zein concentration in the endosperm of ILP and IHP parents, their reciprocal crosses, backcrosses and F_2 progenies. Each value represents the mean of 20 determinations ± s. d.

Endosperm	Generation	Dry weight	Zein	
		mg/endo	mg/endo	% dry weight
ILP (x)		240 ± 11	4.3 ± 0.2	1.8 ± 0.1
IHP (x)		134 ± 5	17.4 ± 1.0	13.0 ± 0.7
ILP x IHP	F_1	230 ± 5	3.9 ± 0.2	1.7 ± 0.1
IHP x ILP	F_1	128 ± 7	18.0 ± 0.3	14.1 ± 0.6
(ILP x IHP) (x)	F_2	260 ± 14	27.8 ± 1.4	10.7 ± 0.4
(IHP x ILP) (x)	F_2	298 ± 12	29.8 ± 2.4	10.0 ± 0.5
(ILP x IHP) x ILP	BC	349 ± 13	32.1 ± 1.8	9.2 ± 0.2
(ILP x IHP) x IHP	BC	298 ± 12	24.7 ± 1.0	8.3 ± 0.3
(IHP x ILP) x ILP	BC	324 ± 11	33.0 ± 2.2	10.2 ± 0.4
(IHP x ILP) x IHP	BC	191 ± 7	17.8 ± 1.0	9.3 ± 0.3

Table 2. Dry weight and protein concentration of the endosperms in ears of F_3 progenies of the ILP and IHP crosses.

Ear	Endosperm weight	Protein concentration
[(IHP x ILP) (x)] (x)	mg/kernel	%
1	190 ± 16	16.8 ± 1.3
2	180 ± 9	13.5 ± 0.7
3	221 ± 10	13.4 ± 0.7
4	271 ± 15	13.3 ± 0.7
5	285 ± 17	13.2 ± 0.9
6	181 ± 10	12.4 ± 0.7
7	259 ± 26	12.3 ± 1.2
8	198 ± 9	11.4 ± 0.6
[(ILP x IHP) (x)] (x)		
1	195 ± 15	18.6 ± 1.3
2	202 ± 21	16.1 ± 1.5
3	207 ± 11	15.7 ± 0.9
4	212 ± 15	15.0 ± 0.9
5	173 ± 11	14.8 ± 0.1
6	194 ± 6	10.3 ± 0.3

Zein Synthesis in Cultured Kernels

ILP kernels had a larger endosperm weight and higher starch/protein (C/N) ratio than the IHP kernels (Table 1); therefore, ILP cultured kernels responded to a high sucrose concentration (350 mM) for maximum kernel development and zein accumulation. Under the high sucrose condition, ILP kernels produced about 6% zein with 88 mM amino acids (Fig. 1) which was at least three times higher than that of the field-grown kernels (1.8%) IHP kernels, on the other hand, were small in kernel weight and had a low C/N ratio (Table 1); therefore, sucrose was not a limiting factor in this experiment for maximum kernel development. Zein concentration in the IHP cultured kernels increased from about 1% to a maximum of about 14% when amino acid concentration increased from 0 to 44 mM (Fig. 2). The small amount of zein produced with zero supplemental amino acids presumably resulted from the endogenous amino acids accumulated during the first 5 DPP grown in the field. These results suggest that the extremely low and high concentrations of zein, 1.8% and 13.0%, produced in the field-grown ILP and IHP plants, respectively, are not controlled exclusively by the endosperm genotypes; in stead, they are attributable, in large part, to the concentration of amino acids provided by vegetative tissues. The concentration of amino acids measured at the mid-silk stage in the leaf and stalk tissues of ILP was 2.6% and 1.6%, respectively, as compared with 3.7% and 3.7% in the leaf and stalk tissues of IHP (Table 3). The vegetative tissues in ILP apparently produced much less amino acids than that of the IHP. Accordingly, ILP accumulated only a small amount of zein in the kernel, indicating the importance of source activity in affecting zein synthesis.

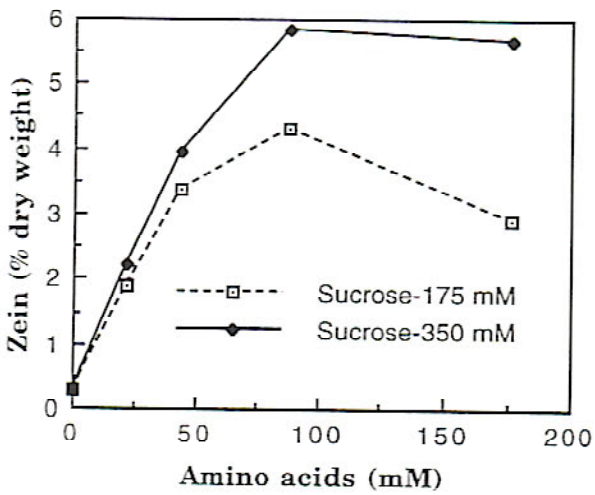

Fig. 1. Effect of amino acid concentrations on zein accumulation in cultured kernels of ILP.

Our previous studies indicate that cultured cells of maize endosperms preserve their ability to synthesize zein proteins (Lyznik and Tsai 1989). Translation of polysomal RNA isolated from cultured cells showed that the pattern of zein polypeptides synthesized *in vitro* was similar to the zein polypeptide composition translated from RNA isolated from the 15 days old kernels. Furthermore, studies with suspension cell cultures indicated that synthesis of zein was regulated by amino acids with glutamine being a major factor in enhancing zein synthesis (Lyznik and Tsai 1989). Thus, although endosperm genes such as *o2* and *fl2* may suppress zein synthesis, under normal conditions, zein synthesis in maize endosperm is regulated by the source supply.

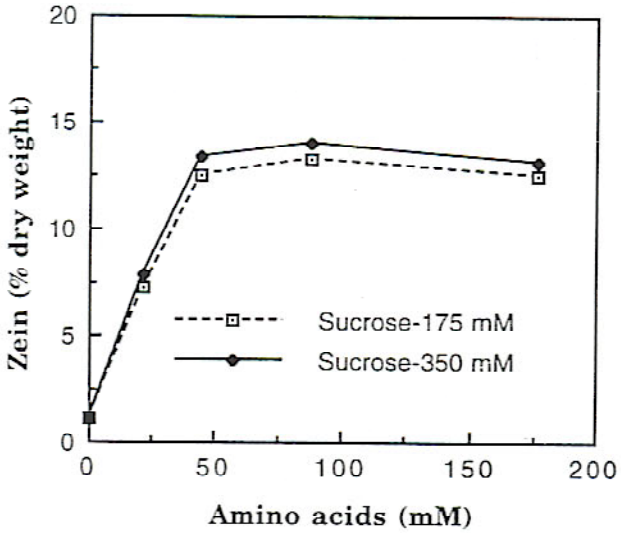

Fig. 2. Effect of amino acid concentrations on zein accumulation in cultured kernels of IHP.

Table 3. Amino acid concentration in leaf and stalk tissues of ILP and IHP harvested at mid-silk stage.

Genotype	Amino acid concentration	
	Leaf	Stalk
	---- % dry weight ----	
ILP	2.6	1.6
IHP	3.7	3.7

Effects of Exogenous N Levels on Zein and Protein Accumulation in endosperms

As shown in Table 4, total protein accumulation in B73 x LH51 endosperms increased in response to N fertility, and the increases in protein were primarily in the form of zein. This observation is consistent with previous studies indicating that unlike other endosperm proteins, zein accumulation responds dynamically to N fertilizer, which makes zein a functional storage house for organic nitrogen (Tsai, 1983). This effect of vegetative source on zein synthesis could be further demonstrated by measuring the accumulation of radioactive amino acid into endosperms incubated with different levels of ^{3}H-glutamine.

Table 4. Zein, non-zein, and total protein concentrations in the endosperm of B73 x LH51 fertilized with different levels of N. Values are mean of 3 replicates ± s. d.

N level	Zein	Non-zein protein	Total protein
kg ha^{-1}	---------- % dry weight ----------		
0	1.20 ± 0.09	4.8	6.0
90	2.50 ± 0.10	5.0	7.5
180	2.80 ± 0.10	5.1	7.9
240	3.60 ± 0.12	5.1	8.7

When the movement of ^{3}H-glutamine was conducted with kernels of active stage of zein synthesis, e.g., 14, 21, and 28 DPP, the glutamine movement into endosperms increased as the concentration of amino acids in the reaction mixtures increased and showed a non-saturation kinetics (Table 5). This result indicates a clear source effect in movement of ^{3}H-glutamine into kernels throughout the period of active zein synthesis and hence the accumulation of a large quantity of zein. Our previous studies have shown that zein synthesis in the endosperm is preferentially stimulated by glutamine (Lyznik and Tsai, 1989). Although the movement of amino acids into endosperm was linearly proportional to the source concentration, the movement of sucrose was stimulated as the concentration of glutamine in the reaction mixture increased from 5 to 80 mM (Table 5), indicating the importance of zein synthesis in affecting starch accumulation in the endosperm.

These studies clearly indicate that accumulation of zein in the kernel is regulated primarily by amino acid substrates provided by the source tissues.

Table 5. Movement of ^{14}C-sucrose and ^{3}H-glutamine into developing endosperms of maize hybrid B73 x LH 51 incubated *in vitro* in reaction mixtures containing 100 mM of ^{14}C-sucrose and varying concentrations of ^{3}H-glutamine. Values are the mean of 5 replicates ± s. d.

DPP	Substrates	Nutrient taken up by endosperms	
	Amino acid	Sucrose	Amino acid
Day	mM	nmoles/endo/hr	
14	80	11.4 ± 0.5	23.5 ± 0.9
14	20	11.3 ± 0.3	5.7 ± 0.2
14	5	9.1 ± 1.0	1.6 ± 0.2
21	80	27.3 ± 2.7	28.0 ± 2.9
21	20	19.4 ± 1.7	8.2 ± 0.9
21	5	10.6 ± 2.0	1.8 ± 0.4
28	80	23.8 ± 2.6	21.4 ± 2.2
28	20	12.3 ± 2.9	4.5 ± 0.9
28	5	6.5 ± 0.8	0.9 ± 0.1

LITERATURE CITED

Association of Official Analytical Chemists. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D.C.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of micrograms of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**: 248-254.

Cully, D. E., B. G. Gengenbach, J. A. Smith, I. Rubenstein, J. A. Connelly and W. D. Park. 1984. Endosperm protein synthesis and L-[^{35}S]methionine incorporation in maize kernels cultured *in vitro*. Plant Physiol. **74**: 389-394.

Lee, L. and C. Y. Tsai. 1984. Zein synthesis in the embryo and endosperm of maize mutants. Biochem. Genet. **22**: 729-737.

Lyznik, L. A. and C. Y. Tsai. 1989. Protein synthesis in endosperm cell cultures of maize. Plant Sci. **63**: 105-114.

Lyznik, L. A., J. Kanabus and C. Y. Tsai. 1989. L-Glutamine and D-glucose uptake by developing endosperms of maize. Phytochemistry **28**: 3271-3275.

Mertz, E. T., R. Jambunatha and S. Misra. 1975. Stat. Bull. **70**: 6-9, Purdue Univ., West Lafayette, IN 47907.

Nelson, O. E. 1979. Genetic control of polysaccharide and storage protein synthesis in the endosperms of barley, maize, and sorghum. Adv. Cereal Sci. Technol. **3**: 41-71.

Reggiani, R., C. Soave, N. DiFonzo, E. Gentinetta and F. Salamini. 1985. Factors affecting starch and protein content in developing endosperms of high and low protein strains of maize. Genet. Agr. **39**: 221-232.

Singletary, G. W. and F. E. Below. 1989. Growth and composition of maize kernels cultured *in vitro* with varying supplies of carbon and nitrogen. Plant Physiol. **89**: 341-346.

Tsai, C. L., I. Dweikat and C. Y. Tsai. 1990. Effects of source supply and sink demand on the carbon and nitrogen ratio in maize kernels. Maydica **35**: 391-397.

Tsai, C. Y., F. Salamini and O. E. Nelson. 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol. **46**: 299-306.

Tsai, C. Y., B. A. Larkins, and D. V. Glover. 1978. Interaction of the *opaque-2* gene with starch-forming mutant genes on the synthesis of zein in maize endosperms. Biochem Genet. **16**: 883-896.

Tsai, C. Y. 1979. Tissue-specific zein synthesis in maize kernel. Biochem. Genet. **17**: 1109-1119.

Tsai, C. Y., D. M. Huber and H. L. Warren. 1980. A proposed role of zein and glutelin as N sinks in maize. Plant Physiol. **66**: 330-333.

Tsai, C. Y. 1980. Note on the effect of reducing agent on zein preparation. Cereal Chem. **57** 228-290.

Tsai, C. Y. 1983. Genetics of storage protein synthesis in maize. In: Janick, J. (ed), Plant Breeding Review. AVI Publishing Co. Westport, CT. **1**: 103-138.

玉米營養組織對 zein 蛋白合成的影響

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摘　　要

本研究的目的在於探討胚乳 zein 蛋白之合成究竟是受控於胚乳基因或營養組織基質之來源，利用兩種玉米品種 (IHP 及 ILP 分別代表 zein 含量高及低的品種) 為材料做交配，並分析 F_1、F_2 及 F_3 子代的種子，發現在 F_2 及 F_3 子代均無 zein 含量之性狀分離。體外種子培養之結果，發現 IHP 與 ILP 種子合成 zein 之能力與培養基中之氨基酸濃度有密切關係。以放射性同位素測定胚乳對氨基酸之吸收，亦發現種子吸收氨基酸以合成 zein 之能力與外界基質之濃度有正相關之影響。這些實驗均證明胚乳中 zein 蛋白之合成量是不受胚乳基因之調控，相反的，營養組織之基質對於此蛋白之合成有密切影響。

關鍵詞：氨基酸，蔗糖，玉米，儲藏組織，營養組織，Zein，*Zea mays* L.。

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