NOTE

Zeins in Sweet Corn (*Sugary-1*)[1]

C. M. WILSON[2,3]

The amount of zein, the major storage protein in corn endosperm, is reduced in seeds with the homozygous *sugary-1* (*su*) mutation, the gene for common sweet corn (Misra and Mertz 1975, Paulis et al 1978, Wilson et al 1989). The gene *su* is linked, but not tightly, to zein genes on chromosome 4 (Wilson et al 1989). Zein is a very heterogeneous family of proteins, consisting of a dozen or more different polypeptides in any one inbred. Zeins can be characterized by isoelectric focusing (IEF) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Wilson 1985, 1986, 1987). Because the major or AB-zeins are deficient in lysine and tryptophan, the nutritional value of corn is affected by the contents of these zeins. Thus it is of interest to determine the changes in regulation of zein synthesis when the *su* mutation is present.

MATERIALS AND METHODS

Mature corn seeds with normal and *su* genotypes (sugary phenotype) in the same background were obtained from several sources. The most detailed studies were made with inbreds Oh43, W64A, and M14, which had been used previously to demonstrate the linkage of the *su* gene to zeins on chromosome 4 (Wilson et al 1989). Other *su* inbreds had been provided to J. S. Wall by the Department of Agronomy, Purdue University. Seven normal inbreds with their *su* versions, which had been mutagenized by ethyl methanesulfonate (EMS) (Neuffer 1982), were provided by M. G. Neuffer, University of Missouri. Two inbreds were provided by D. Doehlert, Agricultural Research Service, National Center for Agricultural Utilization Research (NCAUR).

Powdered endosperm was obtained by using a dental drill bit in a small power drill. The powder was extracted with five volumes of 55% isopropanol with 2% mercaptoethanol (Wilson 1985). After centrifugation, the alcoholic extract was applied directly to an agarose IEF gel, often using 50% more extract from the *su* strains. Serial analysis (transfer of individual IEF zein bands to an SDS-PAGE gel) was performed as previously described (Wilson 1985, 1986).

RESULTS AND DISCUSSION

The *su* gene is a marker for chromosome 4 and is linked to a number of zeins (Wilson et al 1989). However, the IEF zein banding pattern is often unclear, and the intensity of the bands is reduced from those in normal endosperms. The effect of *su* on zein is best shown by the inbred Oh43 (Fig. 1). The zein bands were lighter in the sugary seed than in the normal seed (compare lanes 1 and 2). Especially affected were zeins 10 and 22, with genes located on chromosome 7 (Wilson 1989). Some minor zeins with lower isoelectric points (pI) may be enhanced. Oh43 *su* was crossed with normal N28 (lane 5), giving the reciprocal F1 seeds assayed in lanes 3 and 4. The F1 seeds could not be distinguished visually from normal seeds. The zein patterns were those that would be expected had Oh43 normal (lane 2) been the parent. Zeins 10 and 22 are quite dark in lane 3 (two doses of Oh43 genes) and lighter in lane 4 (one dose). Major zeins in N28 (IEF bands 32, 33.5, and 38) showed the reverse dosage effects. Band 55 did not follow any expected pattern. Oh43 *su* was pollinated by normal Oh43, to give seeds with two doses of sugary and one dose of normal zein genes. The zeins could not be distinguished from the zeins of a fully normal Oh43 seed (not shown). The amount of IEF band 38, a major zein in inbreds W64A and M14 with a gene on chromosome 7, was also reduced in the *su* versions (not shown). A technical problem prevented the use of these inbreds in a genetic test. The conclusion is that the genes for zein are the same in the *su* version of Oh43 as in the normal version, but that the homozygous mutation prevents expression of some genes to the usual extent. Normal appearing seeds contain one to three doses of the *Su* (normal) gene. This dominant gene on chromosome 4 may express a factor needed for activation of zein genes on chromosome 7. This may be similar to the action of the *Opaque-2* gene on chromosome 7, which regulates expression of zein genes on chromosome 4 (Motto et al 1989, Wilson et al 1989).

The other effect of the *su* gene was to induce a new protein at IEF position 30.5 (Fig. 1, lane 1). It was not isolated but is thought to be a zein because it was soluble in alcoholic solutions, had an appropriate pI, and moved on SDS-PAGE to position A1 (Fig. 2). This zein appeared only in homozygous seeds (Fig. 1, lane 1) (Wilson et al 1989). Zein 30.5 was seen in all *su* endosperms examined. These included three *su* versions of field corn inbreds obtained from the Department of Agronomy, University of Illinois, eight sweet corn lines originally obtained from Purdue University, three sweet corn lines used for other studies at NCAUR, and seven *su* lines produced by EMS treatment (Neuffer 1982). The zein patterns of the latter group tended to resemble the corresponding normal versions more than the others, possibly because of the different source of the mutation. Sweet corn lines obtained by spontaneous or induced *su* mutation would be ex-

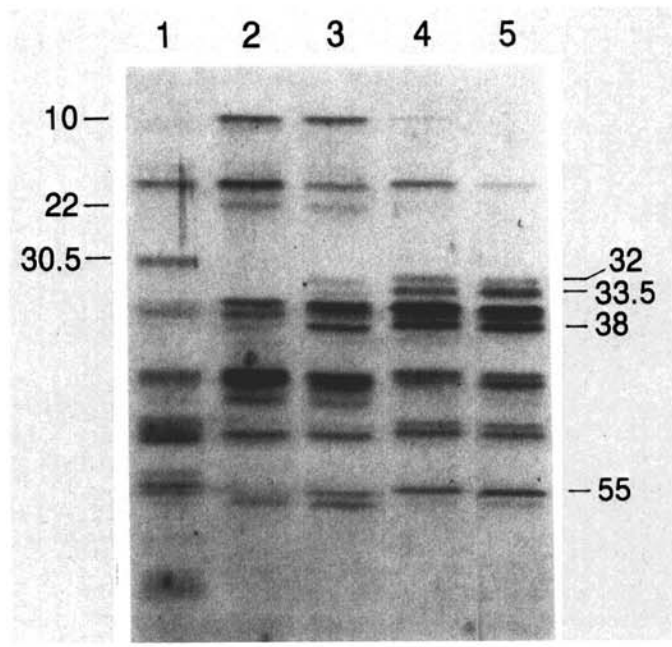

Fig. 1. Isoelectric focusing of zeins from normal(+) and *su* inbreds and their reciprocal crosses. 1 = Oh43*su*, 2 = Oh43+, 3 = Oh43*su* × N28+, 4 = N28+ × Oh43*su*, 5 = N28+. Only no. 1 had sugary phenotype. Anode at bottom.

[1]Presented in part at the AACC 73rd Annual Meeting, San Diego, CA, October 1988.

[2]U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Plant Polymer Research, 1815 N. University St., Peoria, IL 61604.

[3]Current address (not for reprints): 104 W. Pennsylvania Ave., Urbana, IL, 61801.

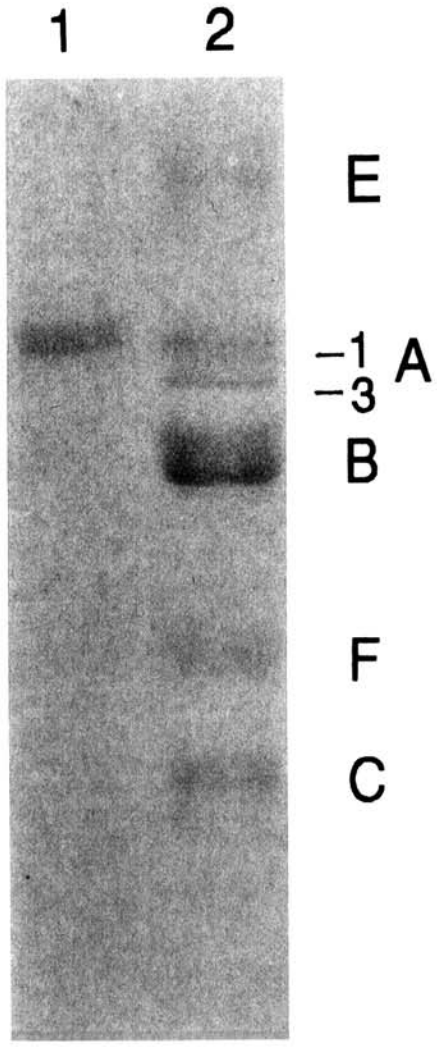

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of W64A*su* zeins. 1 = isoelectric focusing band 30.5, 2 = total zeins, with SDS-PAGE classes indicated. Anode at bottom.

pected to have the same complement of zein genes as the normal inbreds. Some sweet corn inbreds may have been obtained by crossing the inbreds with a different inbred line bearing the mutant gene. The recovered *su* version might have some zeins derived from the original *su* inbred. For example, the gene for IEF band 33.5 is only 3–10 crossover units from the *su* gene (Wilson et al 1989), and its presence or absence would depend upon the number of backcrosses as well as random chance. The intensity of the 30.5 band varied from barely visible to moderate but was weakest in the EMS mutants.

Serial analysis (Fig. 2) of the 30.5 IEF band showed that it may be classed as zein A1/30.5 (SDS-PAGE position/IEF band). Minor zeins C, F, and E are seen in total zein (lane 2). Paulis et al (1990) reported little change in the relative proportions of these zeins in B37 *su* compared to the normal by reversed-phase high pressure liquid chromatography (peaks 1, 3, and 2, respectively).

LITERATURE CITED

MISRA, P. S., and MERTZ, E. T. 1975. Studies on corn proteins. VI. Endosperm protein changes in single and double endosperm mutants of maize. Cereal Chem. 52:161-166.

MOTTO, M., DI FONZO, N., HARTINGS, H., MADDALONI, M., SALAMINI, F., SOAVE, C., and THOMPSON, R. D. 1989. Regulatory genes affecting maize storage protein synthesis. Oxford Surv. Plant Mol. Cell Biol. 6:87-114.

NEUFFER, M. G. 1982. Mutant induction in maize. Pages 61-64 in: Maize for Biological Research. W. F. Sheridan, ed., Plant Molecular Biology Association: Charlottesville, VA.

PAULIS, J. W., WALL, J. S., and SANDERSON, J. 1978. Origin of high methionine content in *sugary-1* corn endosperm. Cereal Chem. 55:705-712.

PAULIS, J. W., BIETZ, J. A., BOGYO, T. P., DARRAH, L. L., ZUBER, M. S. 1990. Expression of alcohol-soluble endosperm proteins in maize single and double mutants. Theor. Appl. Genet. 79:314-320.

WILSON, C. M. 1985. Mapping of zein polypeptides after isoelectric focusing on agarose gels. Biochem. Genet. 23:115-124.

WILSON, C. M. 1986. Serial analysis of zein by isoelectric focusing and sodium dodecyl sulfate gel electrophoresis. Plant Physiol. 82:196-202.

WILSON, C. M. 1987. Proteins of the kernel. Pages 273-310 in: Corn: Chemistry and Technology. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

WILSON, C. M., SPRAGUE, G. F., NELSEN, T. C. 1989. Linkages among zein genes determined by isoelectric focusing. Theor. Appl. Genet. 77:217-226.

[Received July 17, 1991. Accepted October 9, 1991.]