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THE STATUS OF DEVELOPMENT OF MAIZE WITH IMPROVED PROTEIN QUALITY

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This report discusses the analytical and nutritional aspects first, then breeding and genetics. Some of the information is preliminary, and some ideas or conclusions will no doubt be modified or changed as more information becomes available.

In the area of breeding for improved quality protein in maize, tentative conclusions reached so far are: (1) based on lysine levels and feeding trials, opaque-2 seems more promising than floury-2 or the double recessive 0202, fl_2 fl_2; and (2) breeders can use genetic background interactions and more specific modifiers to develop varieties that may be satisfactory in terms of their nutritional advantage, yield and a vitreous kernel type where required for consumer acceptability.

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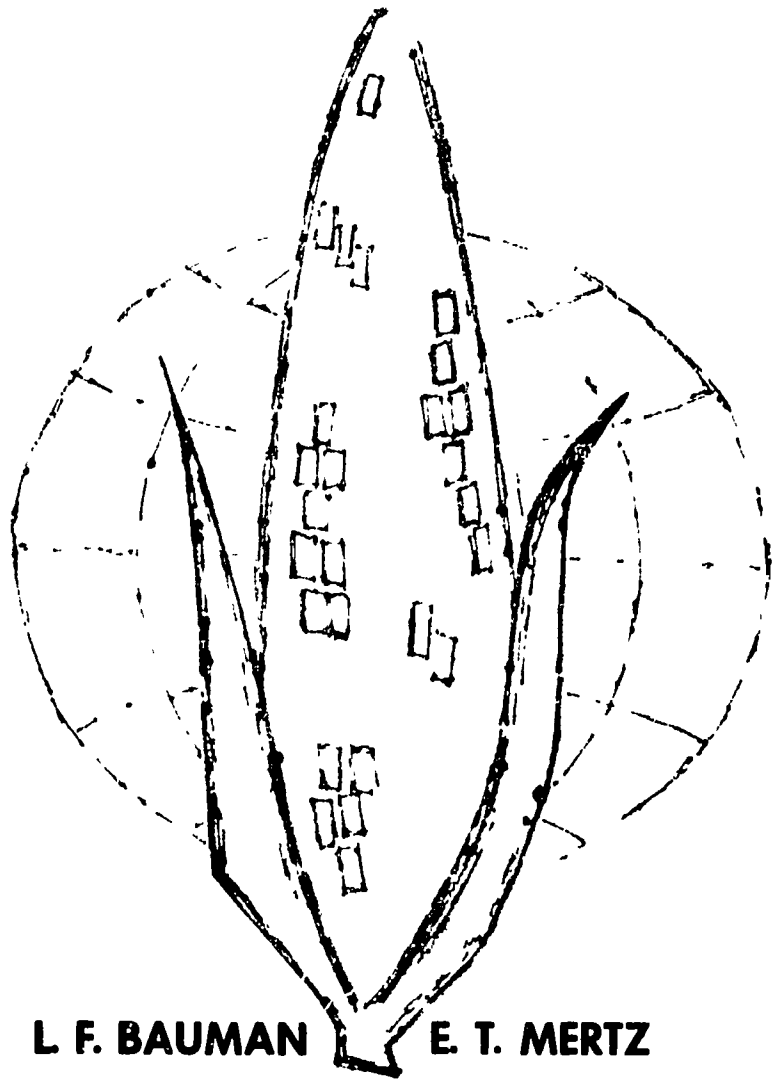

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The Status of Development of Maize with Improved Protein Quality

L. F. Bauman and E. T. Mertz, Purdue University*

The protein in ordinary maize has relatively low nutritional value, and early attempts to improve its quality were both unsuccessful and generally regarded as impractical. However, in 1964, Mertz, Bates and Nelson reported that the opaque-2 gene improved protein quality markedly by increasing the content of the nutritionally-limiting amino acids lysine and tryptophan. Subsequently, they reported that the floury-2 gene was intermediate between normal and opaque-2 and had an increased methionine content.

Nutritional studies that followed confirmed the potential value of opaque-2 maize in helping to solve the world's human and animal nutritional needs. Soon, many maize breeding researchers throughout the world had initiated programs to develop opaque-2 hybrids or varieties.

Today, it is apparent that opaque-2 types must, to some degree, overcome their generally-observed deficiencies when compared with normal maize. These deficiencies include (1) lower yield, (2) greater susceptibility to ear rot, (3) harvest and storage problems, (4) higher moisture content at harvest, and (5) soft starch, which is unacceptable for some areas or uses. To help solve such problems and achieve greater nutritional improvement, there is need for simple and accurate analytical methods for amino acid determination with relatively unsophisticated equipment.

Because of increased breeding work over the past several years and the time-lag associated with publication in scientific journals, it seems appropriate to present here a brief summary of where we are in the development of high quality protein maize. This report discusses the analytical and nutritional aspects first, then breeding and genetics. Some of the information is preliminary, and some ideas or conclusions will no doubt be modified or changed as more information becomes available.

ANALYSIS

Total protein. Total protein is usually determined by the micro-Kjeldahl method. In 1971, ten normal field corn samples submitted to Purdue for analysis varied from 7.3

*Departments of Agronomy and Biochemistry. Supported by the Agency for International Development under a contract entitled, "Inheritance and Improvement of Protein Quality and content in Maize."

to 9.4 percent total protein, with a mean of 8.6 percent. Because of a higher germ-to-endosperm ratio, opaque-2 maize may contain more protein than normal maize.

Amino acids. If a plant breeder needs a complete amino acid profile on certain promising selections, he must use an automatic amino acid analyzer. If, for example, the maize is to be used with legumes, which are low in sulfur amino acids, then he must know the selection's methionine plus cystine content. Or, since high leucine levels interfere with tryptophan and niacin utilization, he may wish to select for low leucine as well as high lysine and tryptophan.

Tryptophan. In most maize samples, colorimetric determination of tryptophan levels in the endosperm is sufficient as an initial screening procedure for protein quality. Endosperm samples containing up to 0.5 grams tryptophan per 100 grams protein are classified as normal quality; samples containing 0.6-1.3 grams may be classified as superior.

Lysine. Usually, lysine value of the endosperm is about 4 times higher than the tryptophan value. For a quantitative lysine value determination, a simple colorimetric method for lysine can be used. Details on the techniques and equipment needed for colorimetric tryptophan and lysine methods and micro-Kjeldahl are available from the Protein Quality Laboratory, Centro Internacional De Mejoramiento De Maiz Y Trigo (CIMMYT), Apartado Postal 6-641, El Batan, Mexico 6, D.F. (Research Bulletin No. 20, May, 1971).

Whole kernel analysis. Simple colorimetric lysine and tryptophan methods seem to work most effectively on the separated endosperm. The maize embroyo has a relatively constant and well-balanced amino acid composition, regardless of genetic background. In addition, the pericarp (or aleurone) may contribute undesirable pigments which interfere with the colorimetric determinations. These factors must be taken into account when whole kernel analyses are desired.

On the average, in ion exchange and colorimetric whole kernel analysis, normal maize gives values of 0.4-0.6 grams tryptophan and 2-3 grams lysine per 100 grams protein, whereas opaque-2 maize gives 0.8-1.2 grams tryptophan and 4-5 grams lysine. Normal endosperm gives values of 0.3-0.5 grams tryptophan and 1.5-2.5 grams lysine per 100 grams protein, while opaque-2 endosperm values measure 0.7-1.2 grams tryptophan and 3-4 grams lysine.

Other rapid procedures, such as the dye-binding method, are being used for screening in some maize breeding programs; but dye-binding does not effectively indicate the precise levels of lysine in opaque-2 segregating populations.

NUTRITION

Studies at Purdue have shown that young white rats fed a 95% opaque-2 maize diet gain nearly four times faster than rats fed a 95% normal maize diet. Tests with weanling pigs produced similar results (Figure 1). It was also found that pigs weighing 125 pounds or more can be fed opaque maize alone without a protein supplement and gain

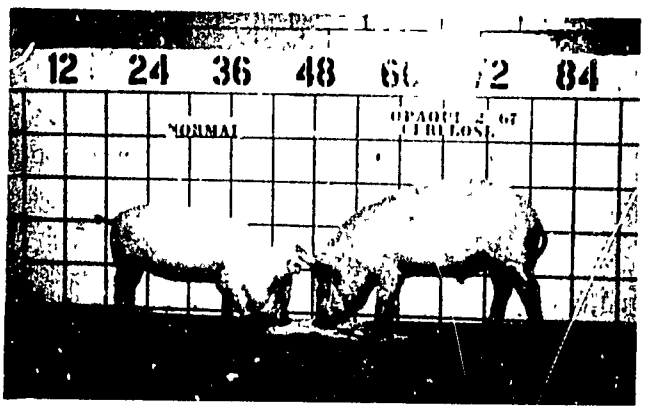

Figure 1. Comparison of swine fed normal and opaque maize as the only dietary source of protein.

as fast as animals on a balanced maize-soybean meal diet, if the ration contains at least 11 percent total protein.

A normal maize diet is usually assumed to be responsible for development of pellagra in humans and a pellagra-like condition in puppies called "black tongue". The reduced level of leucine in opaque-2 maize (about 70% of normal) prevents black-tongue in puppies and should be equally effective in preventing human pellagra, but this has not been tested.

Opaque-2 maize has a high nutritive value for humans. Work with young children in Guatemela and Columbia demonstrates that it is equivalent to skim milk when fed at an adequate level in the diet. It can cure kwashiorkor, a severe protein deficiency disease, when it is the only protein source. Although we have found no published feeding tests on the value of the proteins in immature opaque-2 maize, it is assumed that such maize would be superior to immature normal (roasting ears or on the cob) since the protein differences in these two types can be demonstrated 15 days after pollination.

Nutritional studies on floury-2 maize, although limited, suggest that its response will be in proportion to its level of lysine (based on protein). Usually, the lysine level of floury-2 maize is about halfway between that of opaque and normal. Most all varieties of floury maize observed so far at Purdue are nutritionally inferior to the opaque varieties.

BREEDING AND GENETICS

Backcross Conversion. The immediate approach has been to backcross the opaque-2 gene into inbreds or varieties currently used to produce normal maize. To save time, conversion is generally accomplished by a continuous backcrossing procedure. After the first backcross, the plants are selfed and pedigreed backcrosses are made with the recurrent parent as female. At harvest, the selfed plants are classified as to which are segregating for opaque-2. Then, only the backcrosses to the recurrent parent involving these plants are continued the next generation. Because of the rapid conversion to the recurrent inbred genotype, it is impossible to effectively select for modifiers or interactions.

The opaque-2 gene interacts with different genetic backgrounds. Therefore, as opaque hybrids were reconstituted, some performed poorly with regard to yield and/or grain quality (other than nutritional), while others were more nearly equal to normal hybrids. Yields of the opaque hybrids ranged from 80% to nearly 100% of normal hybrids. Therefore, development of hybrids with satisfactory performance appears feasible. In fact, based on limited use in several countries, farmer acceptance of these "first generation" opaque hybrids has been better than expected.

Introduction of opaque-2 into varieties or synthetics can follow the same procedure used for inbred conversion, except larger populations are required to insure that the germplasm is adequately sampled. This is particularly true of the final backcross. The genetic variations should enable the breeder to visually select for opaque types with better grain quality. Selection for improved performance of opaque genotypes in widebase germplasm of populations offers a promising long-range method to develop acceptable types.

Yield Testing Procedures. Small-plot yield comparisons of opaque and normal maize or check hybrids must involve some control to prevent the opaques from outcrossing with pollen from the normal entries Since the opaque-2 gene tends to be recessive, outcrossing to normal results in normal phenotype and amino acid profile. Hand sibbing of yield test plots is generally not feasible. Therefore, the most practical method is to detassel the normal entries, being careful not to remove any leaf area which would reduce yield. Outcrossing of normal entries with opaque-2 pollen has little or no effect on their yield.

Outcrossing of opaque hybrids to an unrelated opaque pollen source may result in about 10% kernel weight increase and an upward yield bias. This, from a practical standpoint, is difficult to control. Therefore, the researcher must keep this possible bias in mind when interpreting his results. Protein and lysine values are generally not greatly affected by outcrossing to unrelated opaque pollen sources.

Comparisons, of genetically identical sibbed opaque-2 kernels produced on nonsegregating ears and on ears segregating normal kernels have revealed no effect on kernel weight or protein. Therefore, in breeding programs, characteristics of opaque-2 kernels in segregating and homozygous families should be comparable.

Other Effects of Opaque-2. Some additional characteristics of opaque-2 phenotypes are:

1. Percent oil is higher than in normal maize. This is because the germ of opaque types makes up a larger percent of the kernel and is actually heavier than in the normal. Apparently, the reduced starch deposition in the endosperm is compensated for, to some extent, by larger germs.

2. Seedling vigor is generally poorer than normal counterparts, particularly with cold and wet conditions during germination.

3. Cob weight of opaque maize averages about 10 percent less than normal maize.

4. Protein quantity in opaque-2 types generally does not differ significantly from that in their normal counterpart.

Genes Modifying Opaque-2 Phenotype. In 1967, Purdue undertook a population approach to improve opaque-2 which involved recurrent selection. In the program, the selfed segregating families (ears) from a synthetic showing the least difference in kernel weight (expressed as percent between normal and opaque kernels) were selected and intercrossed for the next cycle. After three cycles, the opaque-2 kernel weight expressed as percent of normal was 94.9, compared to 88.5 for the control -- a significant improvement. However, this was disappointing since the improvement was due primarily to reduced kernel weight of the normal kernels. It should be emphasized that little selection for kernel weight *per se* was practiced, and the effect on total yield was not determined.

Another and perhaps more promising approach to modifying the opaque-2 phenotype involves visual selection of more specific modifier genes in segregating families. The kernel expression of the modified types (Figure 2) observed by Purdue, CIMMYT and others might be roughly classified as follows:

1. Vitreous endosperm that is nearly indistinguishable from normal.

2. A 1/2:1/2 phenotype with top or crown part normal or vitreous and the base part opaque. (This is the most frequent occurring type.)

3. Endosperm in which the base part is vitreous and the crown is opaque.

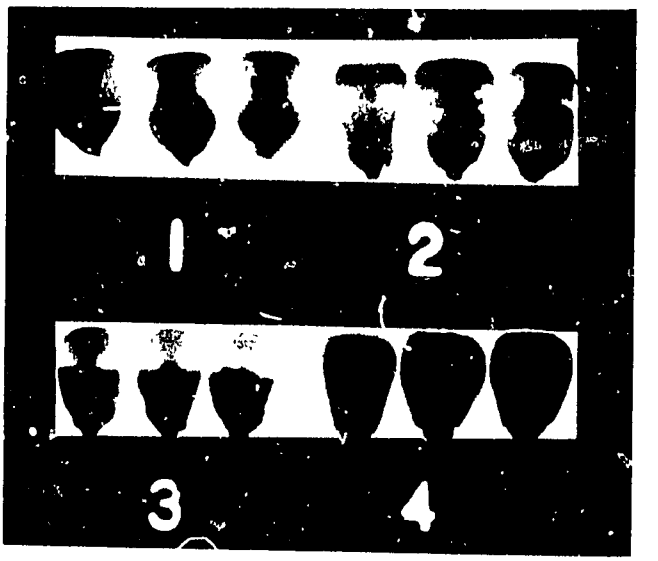

Figure 2. Light transmission with modified opaque types: (1) saddle, (2) diffuse, (3) 1/2:1/2, and (4) standard opaque.

4. A saddle pattern, where the soft or opaque starch is limited to the central portion of the abgerminal side of the kernel.

5. A sectored or diffuse type where soft starch is distributed irregularly in otherwise vitreous starch.

These modifier genes acting on the expression of opaque-2 are heritable, tend to be recessive, probably involve more than one gene, and give somewhat variable expression with various environments. Although good controlled experiments have yet to be conducted, preliminary evidence indicates that lysine content of these modified types is generally satisfactory but probably not as high as in the more opaque phenotypes.

These modifiers mav be useful in providing more acceptable types for certain traditional human food uses and in improving yield and grain quality. Some problems may arise, however, in detecting outcrossing in seed production and in maintaining its opaque identity in market channels.

Potential of Floury-2. Limited tests of floury-2 hybrids indicate that yield reduction compared to normal is not as great as it is for opaque hybrids. Floury-2 grain quality also appears to be somewhat better than opaque-2. In spite of some reports that the floury gene increases protein content, this has not been confirmed under good controlled tests. The main limitation of floury-2 is that lysine values are generally less than for opaque types. Selection of specific modifier genes is also difficult in floury-2, since this gene shows a semi-dominant or additive type of gene action in the endosperm dosage series.

Double Recessive Opaque-2, Floury-2. Early reports indicated the double recessive might give a more normal phenotype than either of the mutants singly. However, this has not been confirmed in subsequent observations; certainly it is not a consistent interaction. Presence of both genes does not mean higher lysine values than opaque-2 types alone. Because of the more complicated breeding procedures and greater difficulty in maintaining pure seed stocks and production with the double mutant, this approach is not highly recommended at this time.

Breeding for Higher Protein Content. The opaque gene tends to maintain improved lysine levels at higher protein content. This makes it feasible, in the long run, to breed opaque varieties with improved protein content and quality. But the job will not be easy, because protein content is influenced greatly by environment and heritability is probably not high. There also may be some negative relationship between yield and protein content.

The correlation between percent protein and percent lysine is positive in opaque-2 maize. But the correlation between percent protein and lysine as a percent of protein is negative. The decision to breed for increased protein content may also be influenced by the availability and use of nitrogen fertilizer in the area concerned.

New High Lysine Mutant Opaque-7. Dr. K. S. McWhirter (University of Sydney, Australia) has found a new endosperm mutant designated opaque-7, which increases lysine and tryptophan in the endosperm. Further studies will reveal the potential value of this new gene.

SUMMARY

At this time the preferred analytical techniques for determining protein content and quality of maize are (1) micro-Kjeldahl for total nitrogen, (2) colorimetric methods for lysine and tryptophan and (3) automatic amino acid analyses for complete amino acid profiles.

In the area of breeding for improved quality protein in maize, tentative conclusions reached so far are: (1) based on lysine levels and feeding trials, opaque-2 seems more promising than floury-2 or the double recessive o_2o_2, fl_2fl_2; and (2) breeders can use genetic background interactions and more specific modifiers to develop varieties that may be satisfactory in terms of their nutritional advantage, yield and a vitreous kernel type where required for consumer acceptability. But breeders are cautioned to not rush the release of maize with improved protein quality until they are reasonably certain of acceptable performance for the specific area.