

## CARTAS AL EDITOR

### **A rapid and simple method to determine maize quality**

Gentlemen:

One of the major problems encountered in the genetic modification of nutritional quality of floury endosperm maize has been the absence of genetic markers to identify high-quality segregants. In the early generations of conversion, each kernel represents a different genetic potential which cannot be measured accurately when a mixture of kernels is tested in the laboratory. The traditional analysis is destructive, and does not allow planting of the same kernels for further generations and selection.

Chemical analysis methods require a laboratory installation and trained personnel which often not available in experiment stations where maize research is conducted (1).. It is important to seek new and simple methods of evaluation without sophisticated equipment, which will allow rapid detection of kernels with improved protein quality in both flint (hard) and floury (soft) phenotypes to improve potential acceptance and consumption by the public and industry (2, 3).

The nutritional quality of maize endosperm protein depends on the concentration of the zein fractions. These segregants or selections with a greater content of zein have a level of lysine and tryptophane, and thus a lower protein quality in the endosperm (4). Properly stained thin sections of endosperm examined under either a light or electron microscope reveal the zein deposited as spherical bodies within the stroma (1, 5, 7). However, these preparations reported in the literature are relatively time-consuming and complicated.

We have worked out simple method for evaluation with the light microscope. Sections are obtained from the cap of the endosperm, with no damage to the germ, by means of a freeze microtome or by hand with a glass knife prepared from a 4 or 5 mm flat glass (Fig. 1). These sections are placed on a glass slide and stained with hematoxylin-eosin, according to the following procedure:

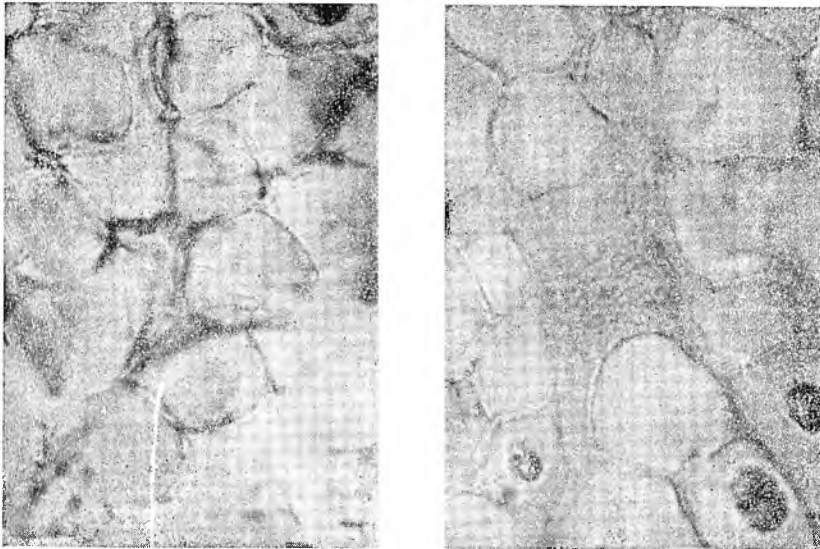
#### Schematic endosperm staining process:

- |   |   |
|---|---|
| 1) Sections of endosperm are obtained without damage to germ. | h) Saturated solution of lithium carbonate or in ammonium solution 2/1000 until a deep blue color develops (plain water can be used for 10 to 20 min. until color development). |
| 2) Sections are placed on glass slide.                        | i) Alcoholic eosin for 15 sec.  |
| 3) Stain.   | j) Alcohol 95° and 100° 1 min. each.  |
| a) Xylol 1 to 2 min.  | k) Clear with xylol.  |
| b) Alcohol 100° and 95° for 1 min. each.                      | l) Permanent mounts can be made.  |
| c) Wash with water.   | m) Observe at 430 or 1000X magnification.   |
| d) Harris Hematoxylin for 15,20 min.                          |   |
| e) Wash with water.   |   |
| f) Two of three dips in acid alcohol.                         |   |
| g) Wash immediately with water.                               |   |

Observations with a light microscope (magnification 1000X) clearly shows the zein bodies. Qualitative classification of protein quality can be determined by the number of these zein bodies present in the stroma and separating the starch cells.

Fig. 2 compares a maize variety with poor protein quality with a large amount of zein deposited between the starch granules, with high quality type. Note the presence and ease of identification of these zein bodies, and the clear indication to the observer of which has the higher quality.

Fig. 2: Microphotographs of two endosperm qualities of soft types. To the left, very high concentration of zein bodies. In the right the same spaces look free of them.



This method is valuable for plant breeders working toward improvement of the nutritional quality of maize in almost any place where the crop is handled. A screening of other types of stains provide a more quantitative system of classification. It is possible that other crops could be evaluated in a similar manner.

*Luz H. Betancur  
Alberto Pradilla  
Charles A. Francis*

Centro Internacional de Agricultura  
Tropical (CIAT). Apdo. Aéreo N<sup>o</sup>  
6713, Cali, Colombia.

## BIBLIOGRAPHY

1. Munck Lars. Improvement of nutritional value in cereals. *Hereditas* 12: 1-128, 1972.
2. Andersen P. P. The feasibility of introducing opaque-2 maize for human consumption in Colombia. CIAT, Technical Bulletin N<sup>o</sup> 1, May 1971.
3. Harpstead D., A. Pradilla. Improving acceptability of opaque-2 maize. VIII International Nutrition Congress. Abstract N<sup>o</sup> H. 12. Aug. 1969.
4. Mertz E. T. High Lysine corn. *Agr. Sci.* 6: 1-6. 1968.
5. Wolf, M. J., U. Khoo. Mature cereal grain endosperm. Rapid glass knife sectioning for examination of proteins. *Stain Techn.* 45: 277-283. 1970.
6. Duvick, D. N. Protein granules of maize endosperm cells. *Cer. Chem.* 38: 374-385. 1961.