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Characterization of South American exotic maize (*Zea mays* L.) populations with opaque phenotype

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ABSTRACT

Eleven exotic populations of opaque maize from Brazil, Bolivia and Chile were characterized. Kernel density, lysine and tryptophan contents, total zeins and non-zeins, zein individual fractions, and soluble sugars were analyzed. Although these populations bear soft endosperm, their zein profiles by gel electrophoresis, the nutritional quality of their proteins as well as the densities of their grains do not follow the patterns present in other opaque maize genotypes. We concluded that these exotic populations are not included in any class of endosperm mutant described so far.

INTRODUCTION

In spite of the relatively high protein content of maize kernels, this cereal is mainly used as a source of energy because its protein is nutritionally unbalanced and inadequate for humans and monogastric animals (Nelson, 1969). This is mainly due to the low lysine and tryptophan content in maize endosperm. Characterization of the *opaque-2* mutant (Mertz *et al.*, 1964) renewed the interest on maize as a protein source. Although this mutant has chalky opaque kernels, with low density, it is high in lysine and tryptophan. Unfortunately, this desirable feature is linked to several undesirable agronomic characteristics (Ortega and Bates, 1983) which have prevented the commercial use of this type of corn.

Plant breeders from the International Maize and Wheat Improvement Center (CIMMYT, Mexico) crossed mutant lines with normal maize populations, and through backcrossing and recurrent selection were able to recover the vitreousness and density of normal lines and maintain the *opaque-2* mutant gene (Vasal *et al.*, 1980). Consequently, the nutritional quality of the original mutant lines was maintained. These new *opaque-2* modified genotypes were designated QPM, for "Quality Protein Maize".

The conversion of the opaque endosperm into vitreous in QPM genotypes was associated with an increase in the accumulation of the 27 kD gamma-zein (Wallace *et al.*, 1990; Paiva *et al.*, 1991; Geetha *et al.*, 1991; Lopes and Larkins, 1991). This correlation as well as the physical and chemical properties of this protein strongly suggest that the 27 kD gamma-zein directly affects the physical properties of the maize grain (Lopes and Larkins, 1991).

By analyzing hard and soft fractions of endosperm from different normal maize genotypes, Dombrink-Kurtzman and Beitz (1993) found strong evidence that endosperm texture is a function of the zein composition in protein bodies. Mature protein

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bodies with high amounts of alpha-zeins are predominant in hard parts of the endosperm whereas "immature" protein bodies with a lower alpha-zein content are more abundant in soft portions of the endosperm (Dombrink-Kurtzman and Beitz, 1993).

MATERIAL AND METHODS

Genetic material

Seeds from two normal maize genotypes BR-106 and BR-201, one QPM (BR-451), and two *opaque-2* mutant lines (UFV-*o2* and IAC*o2* IV) as well as 11 exotic populations were supplied by the CNPMS/EMBRAPA (Brazil). The populations and their origins are as follows: AC-081, AX-001, AX-010, AX-024, MT-II, MT-III, PR-I, and NODZOB UDZA from Brazil; BOL-I and BOL-II from Bolivia; and Preto Chileno from Chile. As the quantity of seeds from the populations was not sufficient for all analyses they were sown in the field and full-sib pollinated.

Kernel density determination

Fifty kernels were put in a 50-ml cylinder and the weight was determined. Absolute ethanol of known density was then added up to the 30-ml mark and the weight was again recorded. Triplicate measurements were made for each genotype and the densities of the kernels were calculated in g/ml according to Kniep and Mason (1989).

Protein and amino acid analyses

For protein, tryptophan and lysine determinations, grains were first immersed in water for 5 min and then the pericarp and germ were excised by hand. The endosperm was dried at 65°C for 16 h, pulverized to a fine powder in a ball mill, and kept at -20°C until use.

Protein content was determined by the micro-Kjeldahl method (Association of Official Agricultural Chemistry, 1980). Tryptophan in the endosperm was determined colorimetricaly by a procedure described by Hernandez and Bates (1969) in which the sample is first hydrolyzed enzymatically with papain. Lysine content was calculated by a correlation with tryptophan concentration according to the equation Lys = 0.3601 + 4.0745 x Trp (Hernandez and Bates, 1969).

Protein extraction

Total protein was extracted from the endosperm powder with 0.0125 M sodium borate, pH 10, containing 1% (w/v) sodium dodecyl sulfate (SDS), and 2% (v/v) 2-mercaptoethanol (2-ME) according to Wallace *et al.* (1990). After centrifugation, non-zeins were precipitated by addition of absolute ethanol to the supernatant to a final concentration of 70% (v/v). After ancther centrifugation, zeins, which remained in the supernatant, were transferred to a new tube and the pellet containing the non-zeins was resuspended in the extraction buffer. Protein concentrations in the fractions were determined by the micro-Kjeldahl method (Association of Official Agricultural Chemistry, 1980).

Gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 7.5 to 17.5% (w/v) gradient gels for separation of zeins and non-zeins. Electrophoresis was basically according to Laemmli (1970); however, high molarity buffer was used in the electrode chambers as well as in the resolving gel (Fling and Gregerson, 1986).

The individual polypeptides in the zein fractions were quantified by densitometry of the corresponding gels in a laser densitometer model Ultroscan XL 2222 (Pharmacia LKB Biotecnology Inc., Piscataway, N.J.).

RESULTS AND DISCUSSION

Kernel density is easily measured, and is directly correlated with vitreousness, hardness, and other agronomically important traits (Pomeranz *et al.*, 1984). In order to verify this type of correlation for South American exotic maize populations bearing opaque kernels (Figure 1), we determined their densities and compared them with those of normal, QPM and *opaque-2* varieties (Table I). As expected, normal and QPM varieties presented higher densities (1.28 g/ml on average) than *opaque-2* varieties and the exotic maize populations (1.11 g/ml on average).

Although the opaque phenotype of the kernels and their low densities suggest that the exotic genotypes are mutants, these mutations do not seem to involve the *opaque-2* locus. This may be deduced by analyzing protein quality, i.e., tryptophan and lysine contents, among the exotic genotypes. They all have

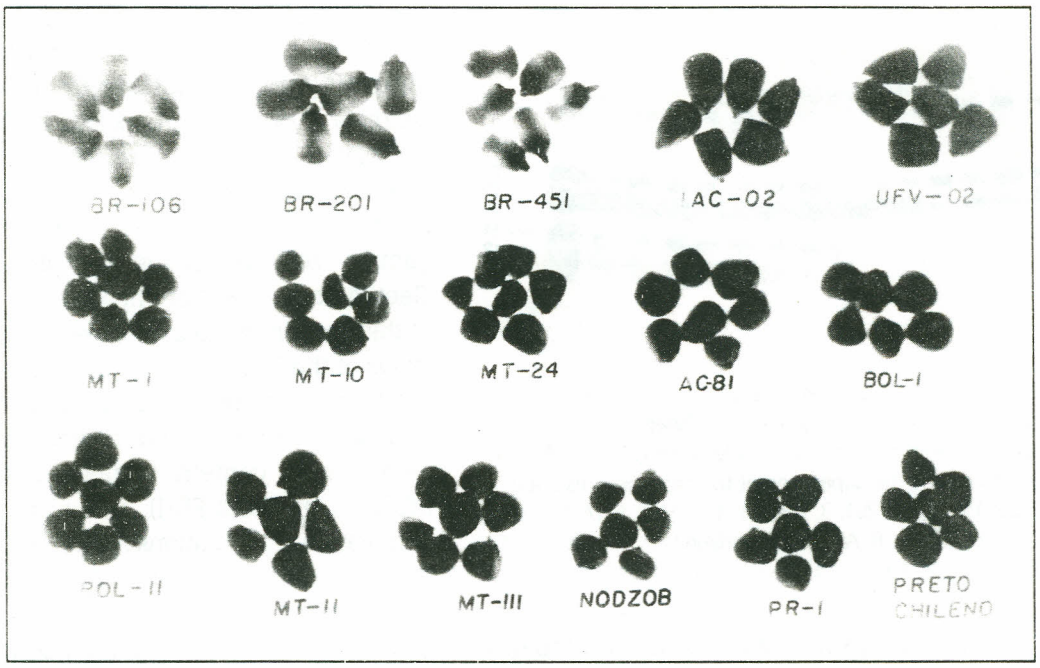

Figure 1 - Photograph of backlit kernels with vitreous and opaque phenotypes.

poor quality, as do normal cultivars BR-106 and BR-201, with 2.4 times less tryptophan and lysine, on average, than the *opaque-2* genotypes (Table II).

The high quality of *opaque-2* and modified *opaque-2* genotypes seem to be due to the lower amount of zeins and the higher amount of non-zeins in the endosperm of these genotypes. Protein determination of the endosperm of exotic genotypes demonstrated that non-zeins are similar to normal genotypes (Figure 2). *Opaque-2* genotypes have extremely reduced amounts of zeins, especially the 22 kD alpha-zein, while in the exotic genotypes they seem unaltered, as in normal maize (Figure 2, lanes 4 and 5, and 6 to 16).

Table I - Densities of normal, QPM, *opaque-2* and exotic maize kernels.

Genotype	Density (g/ml)*
BR-106	1.30 a
BR-201	1.28 a
BR-451	1.27 a
MT-III	1.16 b
PR-I	1.15 bc
UFV-*o2*	1.14 bc
MT-II	1.12 bc
BOL-I	1.12 bc
Preto Chileno	1.11 bc
IAC *o2*-IV	1.11 bc
BOL-II	1.11 bc
AX-001	1.10 bc
AC-081	1.09 bc
AX-010	1.09 bc
AX-024	1.08 bc
Nodzob Udza	1.07 c

*Mean of three separate measurements. Numbers followed by the same letter do not differ by the test of Tukey ($P < 0.01$).

Table II - Tryptophan and lysine contents of normal, QPM, *opaque-2* and exotic maize kernels*.

Genotype	Tryptophan	Lysine
	(% of total protein)	
IAC *02*-IV	1.21	5.28 a
UFV *o2*	1.08	4.74 a
BR-451	0.79	3.59 b
AC-081	0.58	2.73 c
AX-024	0.58	2.73 c
AX-010	0.57	2.67 c
AX-001	0.56	2.64 cd
BOL-I	0.55	2.60 cd
BR-201	0.52	2.48 cd
Nodzob Udza	0.51	2.45 cd
BOL-II	0.50	2.41 cd
PR-I	0.47	2.26 cd
MT-II	0.46	2.25 cd
MT-III	0.43	2.11 d
BR-106	0.43	2.11 d
Preto Chileno	0.41	2.03 d

*Mean of three separate measurements. Numbers followed by the same letter do not differ by the test of Tukey ($P < 0.01$).

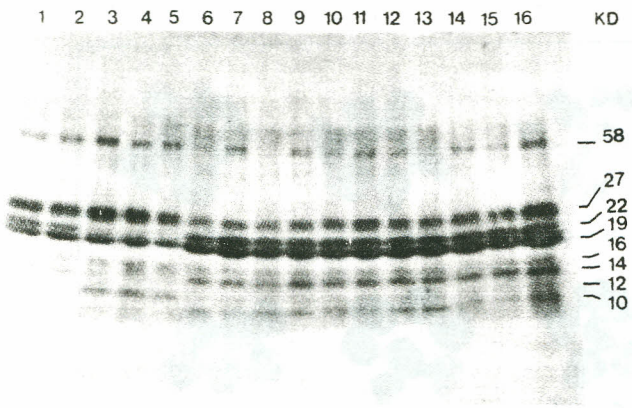

Figure 2 - SDS-PAGE analyses of zeins extracted from the endosperm of different maize genotypes. Twenty-µl aliquots of alcohol-soluble protein extracts were loaded on a 7.5 to 17.5% (w/v) polyacrylamide gel for the separation of the proteins. Lanes are as follows: 1, BR-106, 2, BR-201, 3, BR-451, 4, UFV*o2*, 5, IAC*o2*-IV, 6, AC-81, 7, Preto Chileno, 8, AX-24, 9, Nodzob Udza, 10, BOL-II, 11, MT-II, 12, AX-01, 13, AX-10, 14, PR-I, 15, MT-III, and 16, BOL-I.

The 27 kD gamma-zein is most probably involved with endosperm texture. This protein is located on the periphery of protein bodies in maize endosperm and has a high cysteine content (Lending and Larkins, 1989). In addition, it requires extreme reducing conditions for quantitative extraction, indicating high crosslinking among the cysteine residues (Lopes and Larkins, 1991). These features associated with the observation that conversion of opaque endosperm into vitreous in QPM genotypes was followed by increased accumulation of the 27 kD gamma- zein (Wallace *et al.*, 1990, Paiva *et al.*, 1991, Geetha *et al.*, 1991) prompted us to analyze the levels of this protein in the exotic genotypes. Variation in the levels of the 27 kD gamma-zein in these genotypes did not follow a common trend (Figure 3). However, two groups could be defined, a group with normal 27 kD gamma-zein content (popula- tions BOL-II, MT-II, MT-III, BOL-I and PR-I), and a group with low 27 kD gamma-zein (populations AC-81, AX-10, Preto Chileno, AX-1, Nodzob Udza, and AX-24) (Figure 3). The low amount of this type of zein (20 to 40% less than norma. genotypes) in this latter group might well be related to kernel texture. However, this isolated observation is not sufficient to support any conclusion.

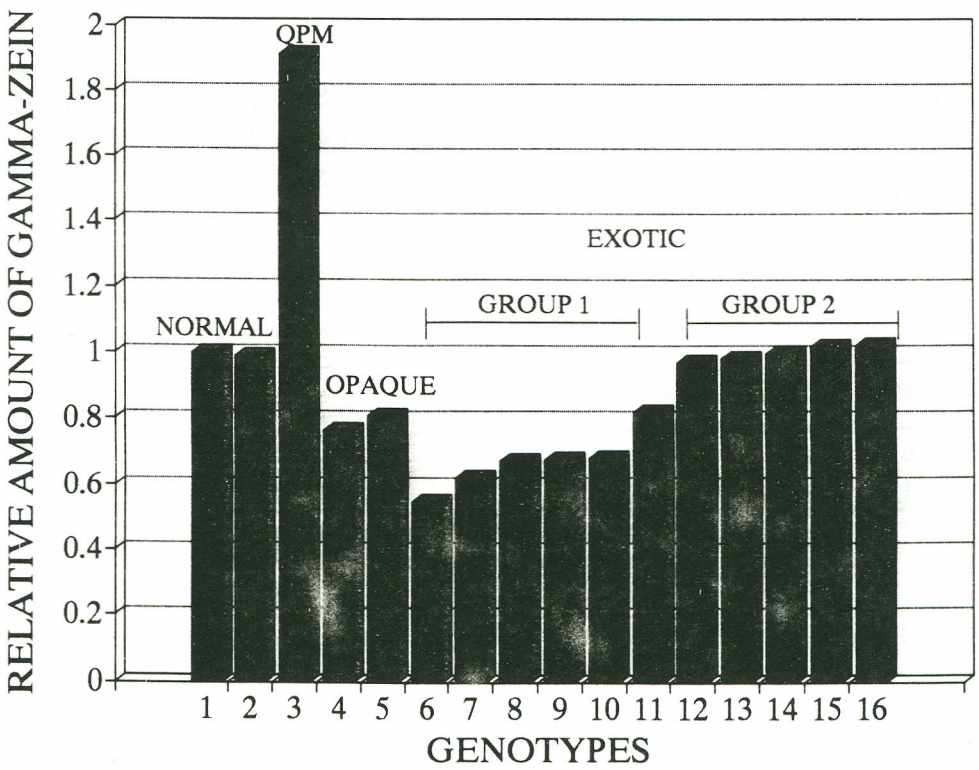

Figure 3 - Relative amounts of the 27 kD gamma-zein in the endosperm of normal, QPM, *opaque-2*, and exotic maize kernels. Total zeins were separated on SDS polyacrylamide gels, which were stained with Coomassie blue, destained, and scanned with a laser densitometer. Each datum point represents the mean of three different scannings. The amount of the 27 kD gamma-zein in normal genotype BR-106 was considered to be 1, and the other values were expressed in relation to it. Genotypes were as follows: 1, BR-106, 2, BR-201, 3, BR-451, 4, UFV*o2*, 5, IAC*o2*-IV, 6, AC-81, 7, Preto Chileno, 8, AX-24, 9, Nodzob Udza, 10, BOL-II, 11, MT-II, 12, AX-01, 13, AX-10, 14, PR-I, 15, MT-III, and 16, BOL-I.

No other difference was detected between the protein fractions of kernels of exotic populations and normal genotypes. For this reason, differences in the starch fraction were also considered (Table III). Several starch mutants which might affect kernel texture have been identified in maize (Dalby and Tsai, 1974). The main feature of these mutants is the presence of large amounts of soluble sugars in the endosperm (Creech, 1965; Tsai, 1979). All exotic genotypes have soluble sugar levels very close to those of normal genotypes and approximately five times less than that of the variety Nutrimaiz, a Brazilan sweet corn, double mutant for *sugary-2* and *opaque-2* genes (Table III).

Maize mutants with low density kernels and poor protein quality, such as *opaque-1* and *floury-1*, have already been identified (Nelson *et al.*, 1965). However, apparently due to lack of commercial value they were not extensively characterized. We believe the opaque exotic genotypes described in this article constitute a new class of mutants with opaque phenotype, low lysine and tryptophan contents, but no significant alterations in the protein or starch fractions. For this reason, further characterization of these genotypes could help the understanding of molecular mechanisms responsible for the determination of endosperm texture in maize.

Table III - Amount of soluble sugars in the endosperm of normal, QPM, *opaque-2*, and exotic maize kernels.

Genotype	% soluble sugars in the endosperm
IAC *o2*-IV	0.94
UFV *o2*	1.09
BR-451	0.72
AC-081	0.85
AX-024	1.13
AX-010	0.97
AX-001	0.69
BOL-I	0.77
BR-201	1.12
Nodzob Udza	0.86
BOL-II	0.81
PR-I	0.81
MT-II	0.71
MT-III	0.85
BR-106	0.69
Preto Chileno	0.60
Mean	0.85
Nutrimaiz	4.88*
Normal	0.82*

*According to Silva *et al.* (1978).

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RESUMO

Onze populações indígenas de milho opaco coletadas no Brasil, Bolívia e Chile foram caracterizadas. Embora essas populações apresentem endosperma macio e farináceo, os padrões eletroforéticos de suas zeínas, a qualidade nutricional de suas proteínas bem como as densidades de seus grãos não seguem os padrões presentes em outros genótipos de milho opaco. Da análise desses parâmetros, conclui-se que as populações de milho indígena estudadas não parecem estar incluídas em nenhuma classe de mutantes para endosperma descritas até o momento.

REFERENCES

Association of Official Agricultural Chemistry (1980). *Official Methods of Analysis*. 13th edn. Washington, pp. 858.

Creech, R.G. (1965). Genetic control of carbohydrate synthesis in maize endosperm. *Genetics 52*: 1175-1186.

Dalby, A. and **Tsai, C.Y.** (1974). Zein accumulation in phenotypically modified lines of *opaque-2*. *Cereal Chem. 51*: 821-825.

Dombrink-Kurtzman, M.A. and **Bietz, J.A.** (1993). Zein composition in hard and soft endosperm of maize and other prolamins. *Anal. Biochem. 70*: 105-108.

Fling, S.P. and **Gregerson, D.S.** (1986). Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. *Anal. Biochem. 155*: 83-88.

Geetha, K.B., Lending, C.R., Lopes, M.A., Wallace, J.C. and **Larkins, B.A.** (1991). *Opaque-2* modifiers increase gamma-zein synthesis and alter its spacial distribution in maize endosperm. *Plant Cell 3*: 1207-1219.

Hernandez, H.H. and **Bates, L.S.** (1969). A modified method for a rapid tryptophan analysis in maize. *Research Bulletin, 13*. CIMMYT, Mexico City, Mexico, pp. 7.

Kniep, K.R. and **Mason, S.C.** (1989). Kernel breakage and density of normal and *opaque-2* maize grain as influenced by irrigation and nitrogen. *Crop Sci. 29*: 159-163.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227*: 680-684.

Lending, C.R. and **Larkins, B.A.** (1989). Changes in zein composition of protein bodies during maize endosperm development. *Plant Cell 1*: 1011-1023.

Lopes, M.A. and **Larkins, B.A.** (1991). Gamma-zein content is related to endosperm modification in quality protein maize. *Crop Sci. 31*: 1655-1662.

Mertz, E.T., Bates, L.S. and **Nelson, O.E.** (1964). Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science 145*: 279-270.

Nelson, O.E. (1969). Genetic modification of protein quality in plants. *Adv. Agron. 21*: 171-194.

Nelson, O.E., Mertz, E.T. and **Bates, L.S.** (1965). Second mutant gene affecting the amino acid pattern of maize endosperm proteins. *Science 150*: 1469-1470.

Ortega, E.I. and **Bates, L.S.** (1983). Biochemical and agronomic studies of two modified hard endosperm *opaque-2* maize (*Zea mays*) populations. *Cereal Chem. 60*: 107-111.

Paiva, E., Kriz, A.L., Peixoto, M.J.V.V.D., Wallace, J.D. and **Larkins, B.A.** (1991). Quantitation and distribution of gamma-zein in the endosperm of maize kernels. *Cereal Chem. 68*: 276-279.

Pomeranz, Y., Martin, C.R., Traylor, D.D. and **Lai, F.S.** (1984). Corn hardness determination. *Cereal Chem. 61*: 147-150.

Silva, W.J., Teixeira, J.P.F., Arruda, P. and **Lovato, M.B.** (1978). Nutrimaiz, a tropical sweet maize cultivar of high nutritional value. *Maydica 23*: 129-136.

Tsai, L.Y. (1979). Early termination of zein accumulation in *opaque-2* maize mutant. *Maydica 24*: 129-140.

Vasal, S.K., Villegas, E., Bjarnason, M., Gelaw, B. and **Goertz, P.** (1980). Genetic modifiers and breeding strategies in developing hard endosperm *opaque-2* materials. In: *Improvement of Quality Traits of Maize Grain and Silage Usage.* (Pollmer, W.G. and Phillips, R.H., eds.). Marinus Nijhoff, London, pp. 37-73.

Wallace, J.C., Lopes, M.A., Paiva, E. and **Larkins, B.A.** (1990). New method for extraction and quantitation of zeins reveal a high content of gamma-zein in modified *opaque-2* maize. *Plant Physiol. 92*: 191-196.

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