Changes in Dry Matter and Protein Fractions During Kernel Development of Quality Protein Maize

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ABSTRACT

Changes in dry matter, protein fractions, and quality during kernel development of quality protein maize (QPM) as a consequence of the interaction between the *opaque*-2 (*o2*) gene and modifier genes were investigated. Protein quality of QPM (i.e., total protein and zein content, tryptophan and lysine levels, and in vitro protein digestibility) was determined at different stages of kernel development for three normal and three QPM populations. Dry-matter accumulations of normal and QPM populations were very similar. QPM contained significantly higher amounts of tryptophan and lysine than did normal maize at early stages of kernel development. Protein-fraction distribution from 14 days after pollination through harvest showed large differences between types of maize, particularly in the prolamin components. Synthesis of zeinlike polypeptides during kernel development followed a characteristic pattern in the QPM endosperm. Isoelectric focusing of the zein and zeinlike fraction during kernel development revealed only minor differences, particularly in the zeinlike components.

Interdisciplinary efforts by plant breeders and biochemists at the International Maize and Wheat Improvement Center (CIMMYT) have resulted in the development of maize germ plasm containing the high-lysine *opaque*-2 gene and modifier genes that favor improved kernel characteristics and agronomic performance (CIMMYT 1985). Quality protein maize (QPM) contains greater amounts of tryptophan and lysine (essential amino acids for humans and monogastric animals) and is now similar to normal maize in agronomic characters. The low yields, higher susceptibility to stored grain insects, and slower drying rate in the field that previously characterized soft *o2* maize have now been largely overcome by recurrent selection for endosperm modifier genes for the *opaque*-2 locus (Vasal et al 1980, 1984). These modifiers change the texture of the endosperm from a soft to a vitreous type while improving grain density and yield.

Maize is an important staple food in many less-developed countries. Around 10% or more of the maize consumed in these countries, particularly in Central and South America and Africa, is harvested at immature stages of kernel development and boiled or toasted. For humans, the superior nutritional quality of the QPM mature whole grain, compared with normal maize, has been well established (Graham et al 1989, Valverde et al 1983), as has its use in food products such as tortillas (Ortega et al 1986) and corn chips (Sproule et al 1988).

Endosperm development after pollination has been studied comparatively in normal and *o2* maize (Murphy and Dalby 1971, Misra et al 1975, Gupta et al 1978, Wall and Bietz 1987) as well as in double-mutant maize germ plasm (Tosello 1975). Developmental changes in endosperm protein of high-lysine mutant cereals have been reviewed by Mertz (1986). The *opaque*-2 gene partially inhibits zein synthesis, with a proportional increase in other protein fractions, so that *o2* maize has about double the amount of tryptophan and lysine in the endosperm protein compared with normal endosperm maize. Since the limiting amino acids for humans and monogastric animals in maize protein are lysine and tryptophan, changes in protein quality of the endosperm protein during kernel development can be evaluated by determining these amino acids.

The purpose of this investigation was to compare the protein quality of QPM and normal populations during kernel development, with particular emphasis on the early periods of maturity (green stage). An additional objective was to obtain information on the effect of the modifiers for endosperm hardness of the *opaque*-2 locus on changes in protein fraction and dry matter accumulation during grain maturation.

MATERIALS AND METHODS

From CIMMYT's maize breeding program, normal endosperm maize populations 25 (Blanco Cristalino-3), 27 (Amarillo Cristalino-1), and 43 (La Posta), and three QPM populations of similar grain type and maturity that had been selected for endosperm hardness and protein quality for several cycles-populations 62 (White Flint QPM), 63 (Blanco Dentado-1 QPM), and 65 (Yellow Flint QPM)-were planted in Mexico at Tlaltizapan, Morelos (19° N, 940 m above sea level), during the 1987B cycle. Although the QPM populations were not isogenic (conversions of the normal populations used for comparison and their genetic backgrounds were in some cases quite different), their grain type and maturity were similar to those of the normal populations, which were the best ones available at CIMMYT for each class of germ plasm. The experimental design was a type of split-plot arrangement, with grain type as the main plots and QPM populations and normal populations as subplots. The main plots were arranged in randomized complete blocks, with four replications. Each subplot had four rows 5-m long. Plant density was about 53,000 plants per hectare. All plants in the plots of the normal endosperm populations were detasseled to avoid contamination of the QPM populations with normal pollen. Silking dates were recorded, and for each replication and population three ears were sampled from bordered plants at 14, 21, 28, and 35 days after pollination and at harvest about 55 days after pollination. Samples were sent to the laboratory for analysis. Twenty developing grains from each ear were randomly collected and freeze-dried and weighed to give 60 grains from three ears per population and replication.

Change in dry matter accumulation was recorded by measuring 100-grain weight. The average of the four replicates was used to obtain dry matter accumulation curves. Kernel density was determined in duplicate for harvested samples by the conventional water displacement method (Brown 1962).

For biochemical analyses, the grain samples were finely ground in a Udy mill with a 0.4-mm mesh and defatted with hexane in a Soxhlet-type continuous extractor. All chemical parameters were determined at least in duplicate. Protein content was determined colorimetrically (Hofstader 1966), using an autoanalyzer standardized with micro-Kjeldahl values (AOAC 1980) and a conversion factor of 6.25. Tryptophan content was determined with the modified colorimetric method (Villegas et al 1984). Zein content was determined by a turbidimetric method (Paulis et al 1974). Lysine content was determined using ion-exchange chromatography (Moore and Stein 1951) after acid hydrolysis for all stages of kernel development in samples of the normal population La Posta and the QPM population Blanco Dentado-1, which

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were of similar grain type, maturity, and genetic background. The same populations were analyzed for pepsin in vitro digestibility (Axtell et al 1981) using casein as the reference protein. Protein fractionation, using the Landry-Moureaux (1970) scheme, was performed on endosperm samples from the normal and QPM populations (prepared by dissecting developing seeds) taken 14 and 28 days after pollination and at harvest, about 55 days after pollination. Five different protein fractions were quantified: I (albumins, globulins, and soluble nitrogen), II (true zein), III (zeinlike), IV (glutelinlike), and V (true glutelins), and a residue. Changes in distribution of these proteins during kernel development were compared. The zein and zeinlike protein fractions during endosperm development were studied in more detail by separating the components present in each fraction by isoelectrofocusing in ultra-thin 6*M* urea polyacrylamide gel in the 5–9 pH range (Righetti 1983), as described in the LKB (1986) manual. Qualitative patterns were compared for the normal and QPM populations at different kernel development stages.

RESULTS AND DISCUSSION

Physical and Agronomic Characteristics

The rates of dry matter accumulation recorded for normal and QPM populations did not differ significantly (Fig. 1). In contrast to reports comparing soft *opaque*-2 and normal maize (Glover 1976), only one QPM population showed lower dry matter at harvest than the normal population used for comparison. Comparisons indicate that yields of several of these QPM populations are equal to those of normal populations (Bjarnason et al 1988). Kernel density values for the QPM populations under study were similar to those of the normal populations (Table I). The improved kernel type of the QPM populations, which makes them phenotypically indistinguishable from normal materials, has apparently resulted in improved rates of starch deposition in the grain and kernel density similar to normal endosperm types.

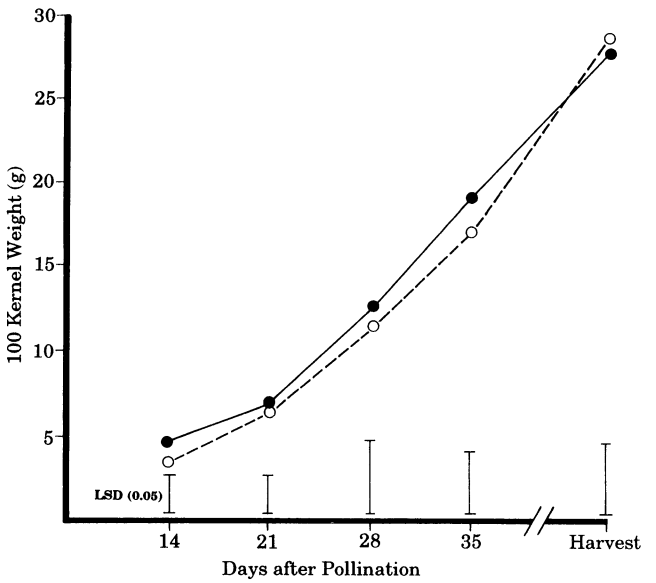

Fig. 1. Kernel dry matter accumulation during kernel development in three normal (○) and three quality protein maize (●) populations.

TABLE I
Grain Density at Maturity of Normal Endosperm and Quality Protein Maize

Grain Type/ Population	Grain Density[a] (g/ml)
Normal	
Population 25	1.272
Population 27	1.255
Population 43	1.262
Mean	1.263
Quality protein maize	
Population 62	1.255
Population 63	1.240
Population 65	1.245
Mean	1.247

[a] Standard errors of difference between pairs of means, at 18 df, were 0.023 within each population group and 0.014 between means of the normal and quality protein maize populations.

Biochemical Analyses

Protein content followed the same decreasing trend during kernel development in normal and QPM populations (Fig. 2). Values decreased from around 14.6% at 14 days after pollination to 9.0% at maturity.

Differences in protein content among populations and types of germ plasm were larger at the initial stages of kernel development and smaller at harvest. The decline in protein content (due to rapid starch synthesis and enzymatic nitrogen breakdown) was similar to that observed for normal maize and *o*2 germ plasm during kernel development (Misra et al 1975). Nevertheless, QPM populations showed slightly lower protein values at harvest than normal populations, as has been reported with some soft *o*2 genotypes (Wall and Bietz 1987).

QPM followed a zein biosynthetic trend (Fig. 3) similar to that reported for soft *o*2 endosperm materials (Wall and Bietz 1987). Zein synthesis was drastically reduced from the initial stages of kernel development and never reached more than 10% of the total protein in the developing grain. Typical of QPM materials was the drop in zein content during the final stage of development before harvest, probably due to the synthesis of other types of endosperm proteins.

Tryptophan contents of QPM and normal populations did not follow the amounts of zein proteins, particularly after 21 days after pollination (Figs. 3 and 4). In QPM germ plasm, the amount of tryptophan in the protein increased to around 0.92%, whereas in normal samples it decreased continuously from 21 days after pollination until harvest, reaching values as low as 0.56%.

Lysine contents of normal population 43 and the QPM population 63, both of Tuxpeno background, also did not follow the amounts of zein proteins (Figs. 3 and 5). The percentage of lysine in protein decreased during kernel development in both populations, with the values for QPM always superior to those for normal materials. Lysine content also declined during rapid zein synthesis in normal maize (e.g., between 21 and 35 days after pollination). The drop in lysine content of endosperm protein did not correlate with tryptophan content. In the normal popu-

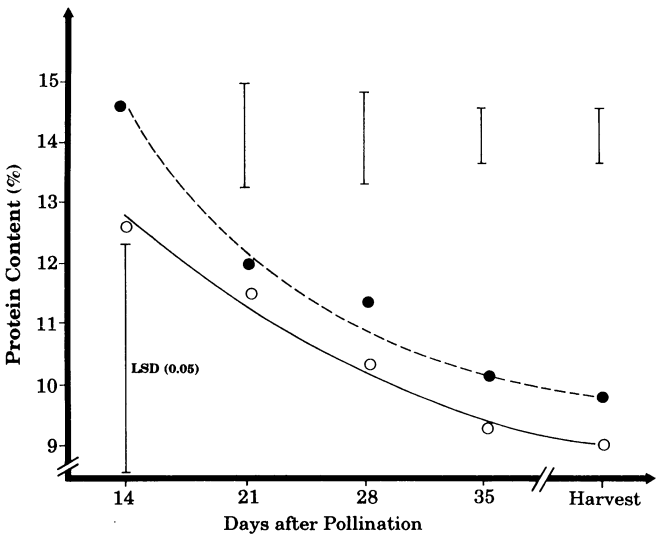

Fig. 2. Changes in protein content of three populations with normal endosperms (●) and three populations of quality protein maize (○) during kernel development.

lation, even though the drop in lysine was considerable, tryptophan declined only slightly. In contrast, the lysine in the QPM sample decreased and the tryptophan concentration increased. Significantly higher levels of lysine and tryptophan were observed at all stages of maturity in the QPM sample, with the maximum benefit at grain maturity.

In vitro pepsin digestibilities (Fig. 6) of both normal and QPM samples decreased during kernel development by approximately 20%. No significant differences were detected between the QPM and the normal population during kernel development.

The percentages of three protein extractions from endosperm samples are shown in Figure 7. At 14 days after pollination, the predominant proteins in both normal and QPM endosperm were the albumins, globulins, and soluble nitrogen present in fraction I. Their concentration diminished throughout endosperm development. The amounts of fraction I components in the QPM population were almost double those in the normal population. Con-

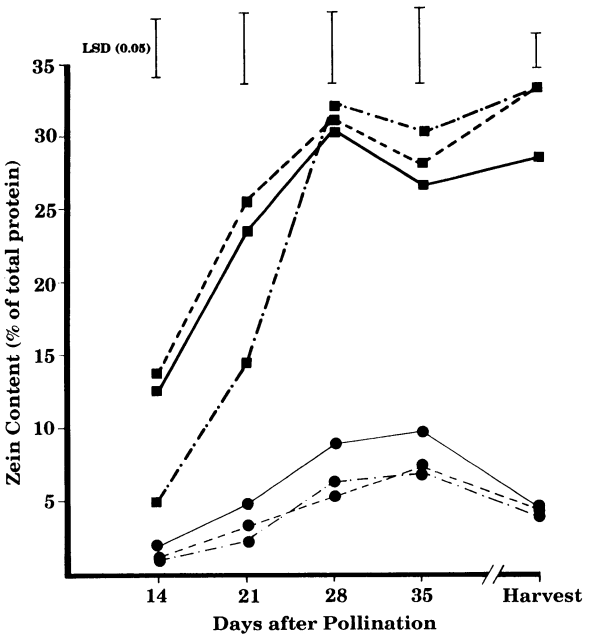

Fig. 3. Zein biosynthesis during kernel development in three populations each of normal and quality protein maize maize (QPM): population 25 normal (■——■), population 27 normal (■— —■), population 43 normal (■—·—■), population 62 QPM (●——●), population 63 QPM (●— —●), population 65 QPM (●—·—●).

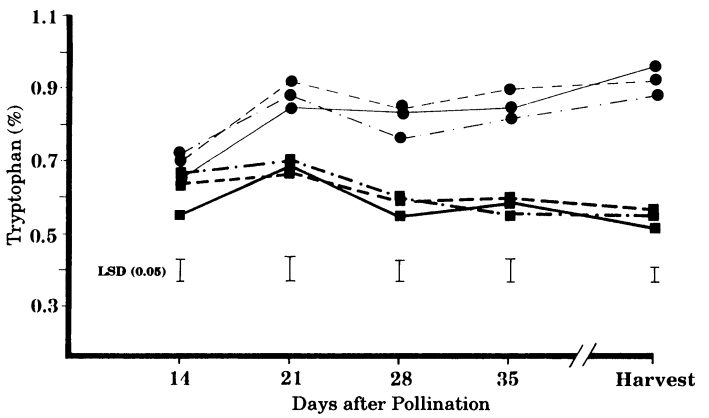

Fig. 4. Changes in tryptophan content in the whole grain protein during kernel development in three populations each of normal and quality protein maize maize (QPM): population 25 normal (■——■), population 27 normal (■— —■), population 43 normal (■—·—■), population 62 QPM (●——●), population 63 QPM (●— —●), population 65 QPM (●—·—●).

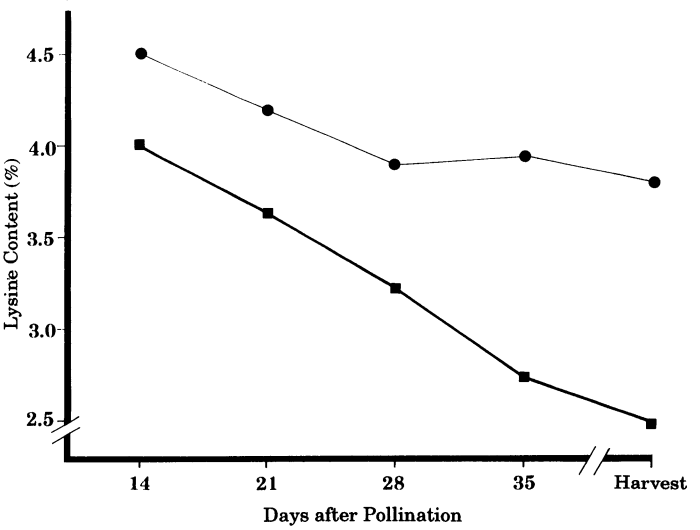

Fig. 5. Changes in lysine content in the whole grain protein during kernel development in normal endosperm population 43 (■) and quality protein maize population 63 (●). The difference between the lysine content of populations 43 and 63 was significant at all harvest dates, and the difference between the slopes of the two populations was significant at $P = 0.01$.

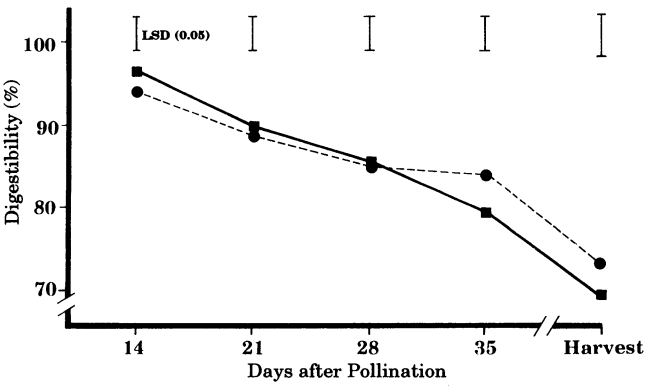

Fig. 6. Changes in in vitro protein digestibility during maize kernel development in normal endosperm population 43 (■) and quality protein maize population 63 (●).

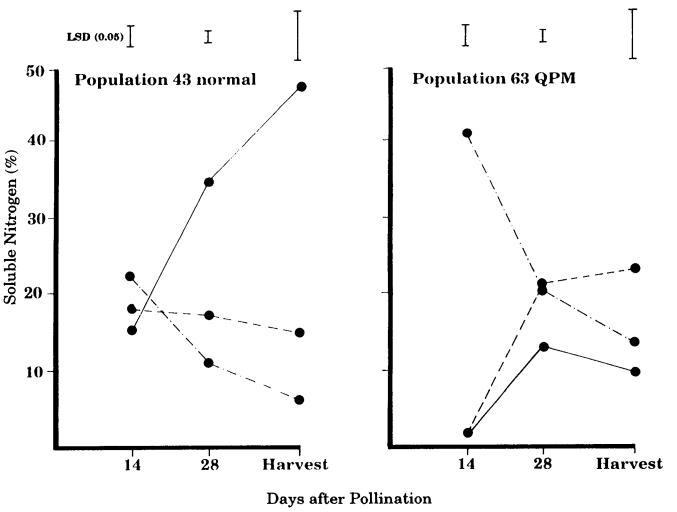

Fig. 7. Changes in distribution of albumins and globulins (●—·—●), true zein (●——●), and zeinlike protein (●— —●) during kernel development in endosperm samples from a normal and a quality protein maize population.

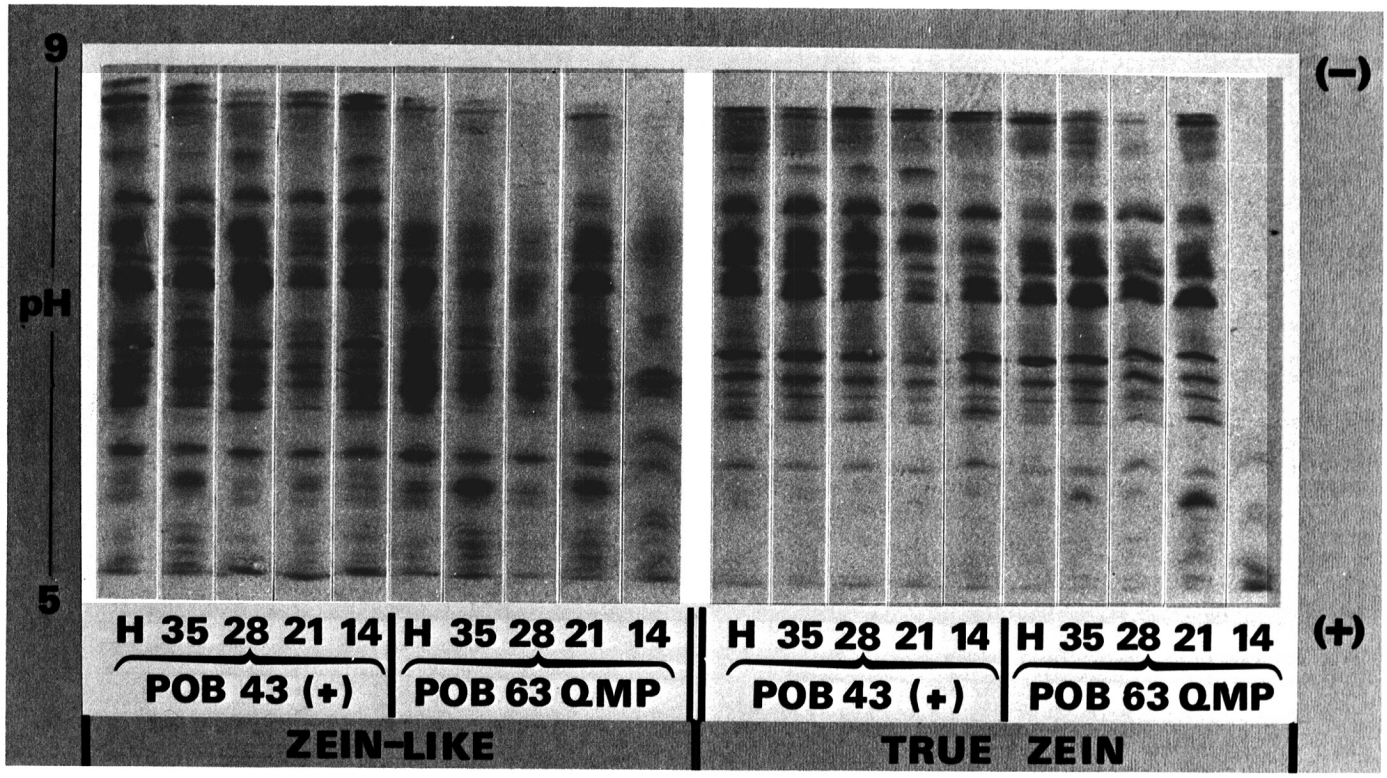

Fig. 8. Isoelectric focusing patterns of zein and zeinlike components during maize kernel development (14, 21, 28, and 35 days after pollination and at harvest) in population 43 (normal) and population 63 (quality protein maize).

centrations of fraction I components in mature grain were within the range of values reported for normal maize (Misra et al 1975) and for QPM (Ortega and Bates 1983). Using a different fractionation scheme, Wall and Bietz (1987) recovered a higher content of fraction I components. This suggests that the extraction procedure used in this study may not be ideal for completely solubilizing the high concentration of albumins and globulins present at this early stage of endosperm development.

Amounts of true zein were extremely low at the initial stages of kernel development in QPM, whereas normal maize rapidly initiated synthesis and accumulated zein (Fig. 7).

The synthesis of the zeinlike protein fraction in the QPM materials (Fig. 7) followed a completely different trend from that in normal genotypes and from previous reports for *o*2 soft endosperm genotypes (Wall and Bietz 1987). Zeinlike components increased 10-fold, from a concentration similar to the true zein components at 14 days after pollination to a concentration about double the true zein components at 28 days after pollination. In the normal population, zeinlike components remained constant during kernel development. The pattern of zeinlike synthesis in QPM may be a consequence of the interaction between modifier genes and the *o*2 locus. Modifier genes of the *o*2 locus resulting in a harder endosperm have been related to increased synthesis of zeinlike proteins (Gentinetta et al 1975, Ortega and Bates 1983).

QPM endosperm contains higher amounts of glutelins than normal endosperm due to the *o*2 gene effect. This difference was manifested from 14 days after pollination, mainly in fraction V (true glutelins). During kernel development, the proportion of this protein in the endosperm significantly increased in QPM, whereas no trend was detected in normal maize (data not presented here).

Total soluble nitrogen recovery upon protein fractionation for both populations at 28 days after pollination was considered adequate, since only 5–9% of the total nitrogen from the original endosperm samples remained as residue. The residue from mature grain of both populations was approximately 22% of the total original nitrogen content.

Isoelectrofocusing was used to characterize zein and zeinlike fractions and to determine whether different components were synthesized at different stages or higher concentrations of the same components. Changes in the protein pattern for zein and zeinlike extracts during kernel development were minimal and probably confined to quantitative changes in the zeinlike components, particularly in the QPM genotype. Protein patterns in the QPM material differed from those in the normal material in the absence of certain components (Fig. 8). This was previously reported to occur in mature kernels as a consequence of the *o*2 gene effect (Righetti et al 1977; Wilson 1984, 1985).

From 15 to 18 similar zein and zeinlike components were detected in both normal and QPM samples. In contrast, 10 zeinlike polypeptides (with acid isoelectric points) were not detected in the true zein protein fraction or were present only at very low concentrations in both genotypes.

CONCLUSIONS

Selection for modifier genes for the *o*2 locus has led to QPM populations that have dry matter accumulation curves, kernel density, and vitreosity similar to those of normal endosperm maize populations. At the early stages of kernel development (21–28 days after pollination), the normal populations had on average 1% higher protein content than QPM; the difference was less at harvest. Tryptophan content increased in the QPM populations during kernel development, whereas it decreased in the normal populations. Lysine decreased in both types of germ plasm. The initially higher content of fraction I components in QPM materials, in addition to the much lower synthesis of total zein (devoid of lysine), resulted in a higher content of lysine at the "green" stage and in mature grain.

As a consequence of the *o*2 gene, QPM showed partial suppression of zein synthesis, manifested by very late initiation and a lower biosynthetic rate of zein and zeinlike proteins during kernel development. A particularly high quantity of zeinlike components accumulated in QPM germ plasm.

Protein digestibility showed no significant difference between normal maize and QPM in all stages of kernel development. The higher content of tryptophan and lysine in QPM during all stages of kernel development suggests that the protein quality of QPM is also superior at all stages of kernel development.

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