

HETEROGENEITY OF *Coix*, MAIZE, AND TEOSINTE PROLAMINS DETECTED BY ISOELECTRIC FOCUSING

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ABSTRACT

The prolamins of maize, teosinte, and *Coix* strains were extracted and analysed by isoelectric focusing. Each strain analysed showed a distinct prolamins profile. The extensive charge heterogeneity observed in the *Coix* profiles suggests that multiple genes encode for the prolamins, as previously reported for maize and teosinte prolamins. Among the prolamins bands that characterize the maize genotypes, a group of four bands with pI between 6.55 and 6.27 and a band with pI 5.56 were found in every maize strain. Some of the teosinte strains analysed also had bands distributed in the pI range of 6.55 to 6.27. The band with pI 5.56 was observed in every teosinte strain analysed. Based on the observed homologies, it can be inferred that the isoelectric focusing profiles of the maize strains are more homologous to the profiles of teosintes than to those of *Coix*.

INTRODUCTION

Zein, the major protein of maize (*Zea mays* L.), is a group of prolamins that represents more than 50% of the total endosperm protein. Those prolamins have distinct physical properties and can be grouped into α , β and γ -zeins (Kodrzycki *et al.*, 1989). When subjected to sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE), the zein proteins are subdivided into five major classes with apparent molecular weights of 22 and 19 kDa (α -zeins), 14 kDa (β -zein), 27 and 16 kDa (γ -zeins), and 10 kDa (δ -zein) (Larkins *et al.*, 1984; Kodrzycki *et al.*, 1989). In isoelectric

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focusing (IEF) gels, zein shows a complex band pattern (Gianazza *et al.*, 1977) that is specific for each maize genotype analysed (Righetti *et al.*, 1977; Soave *et al.*, 1978a).

The α -zeins are responsible for most of the charge heterogeneity observed for the protein in IEF gels (Heidecker and Messing, 1986). It has been suggested that the charge heterogeneity is a consequence of amino acid differences in the repetitive sequences of the α -zeins (Heidecker and Messing, 1986). The zein profile complexity observed in IEF analysis is well correlated with the heterogeneity observed for the sequences of zein cDNA clones (Galili *et al.*, 1987). The charge heterogeneity observed for α -zeins suggests that multiple genes encode for these proteins (Marks and Larkins, 1982).

In contrast to α -zeins that exhibit extensive charge heterogeneity in IEF gels, the β and γ -zeins seem to consist of only one or two polypeptides, which is in agreement with the detection of one or two genes that code for those proteins in the maize genome (Larkins *et al.*, 1984; Kirihara *et al.*, 1988). Esen *et al.* (1981) reported 14 to 16 polypeptides for the 27 kDa γ -zein when the protein was separated in polyacrilamide IEF gels with ampholines with pH from 6 to 9.5 and 6 M urea. However, the 27 kDa γ -zein IEF components do not seem to be coded by a multi-genic family since the data obtained from the sequence of seven 27 kDa DNA clones suggests that only one sequence codes for the protein (Wang and Esen, 1986).

Isoelectric focusing of zein proteins has been widely employed in the characterization of maize inbred lines (NuCCA *et al.*, 1978). Such characterizations make it possible to detect contaminations between different inbred lines. Zein IEF profiles also have been used in taxonomic studies (NuCCA *et al.*, 1978) and in genotype comparison studies (Wilson, 1986).

The objective of the present study is to determine the heterogeneity of maize, *Coix*, and teosinte prolamins by IEF. The IEF pattern complexity of different genotypes reported here provides information about the relationships of maize and related grasses.

MATERIALS AND METHODS

Plant material

The plant material used in the experiments came from members of the tribe *Andropogoneae*, including *Zea mays* L. var. Maya and inbred lines L1038, L126-3, L107-1, and L75-3, teosinte strains *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes), *Zea mays* L. ssp. *mexicana* (Batan), *Zea mays* L. ssp. *mexicana* (Central Plateau), *Zea mays* L. ssp. *luxurians*, *Zea mays* L. ssp. *mexicana* (Balsas), and *zea perennis* and *Coix lacryma-jobi* L. strains Adlay, Acre, Faxina, Rosário and Tailandia. The plant material used was obtained from the stocks of the Maize Genetics Laboratory of the University of Campinas.

Protein Extraction

About 500 endosperms from each of the maize, *Coix*, and teosinte strains were ground to a powder in a ball-mill and defatted with acetone. The samples (about 100 mg) were extracted overnight with a 55% (v/v) aqueous isopropanol solution containing 2% (v/v) 2-mercaptoethanol. A 5:1 (solvent:solid) ratio was used. After centrifugation in a microfuge for 10 min, the supernatant containing the prolamins was collected and stored at -20°C .

Isoelectric focusing

The isoelectric focusing of prolamins were conducted as described by Wilson (1984) for zein. The gels were comprised of 1% agarose, 1.6% ampholine pH 5-8, 0.4% ampholine pH 3.5-9.5 (LKB), 6 M urea, and 2 mM dithiotreitol (DTT). Protein samples were applied to slots near the anode of the gels. After focusing, the gels were fixed with acetic acid/trichloroacetic acid/water (5:3:92, v/v/v). The gels were stained with 0.5% Coomassie Brilliant Blue R 250 in methanol/acetic acid/water (20:10:70, v/v/v) and destained in methanol/acetic acid/water (20:10:70, v/v/v). The isoelectric points of the prolamin polypeptides were determined according to Sigma specifications for IEF pI markers (Sigma, IEF-M1).

RESULTS

The total prolamin extracts from maize, *Coix*, and teosinte showed a complex band pattern in IEF gels containing a mixture of ampholines pH 5 to 8 and pH 3.5 to 9.5. The IEF patterns were unique for each maize, teosinte and *Coix* material analysed.

Nine major bands were detected in the prolamin IEF pattern of *Coix lacrymajobi* L. var. Acre (Figure 1). The nine bands were distributed in a pI range of 8.26 to 6.34. For *Coix lacryma-jobi* L. var. Adlay (Figure 1); seven major bands were detected, distributed in a pI range of 7.76 to 6.20. Although the IEF patterns of Adlay and Acre strains of *Coix* were different, there were many polypeptides in the two strains that exhibited the same pIs in the gels: 7.76, 7.62, 7.26, 6.91, 6.55, and 6.34. However, some of these common pI bands showed different intensities in the IEF gels. The greatest intensity difference was observed for the band with pI 6.91, which appeared to have three times more protein in Acre than in Adlay. Apparently, the gene that codifies for the pI 6.91 polypeptide in Acre is either an overexpressing or a duplicated gene.

The IEF pattern of the maize inbred line L1038 (Figure 1) showed seven major bands, distributed in a pI range of 7.26 to 5.56. The IEF pattern of L1038 was

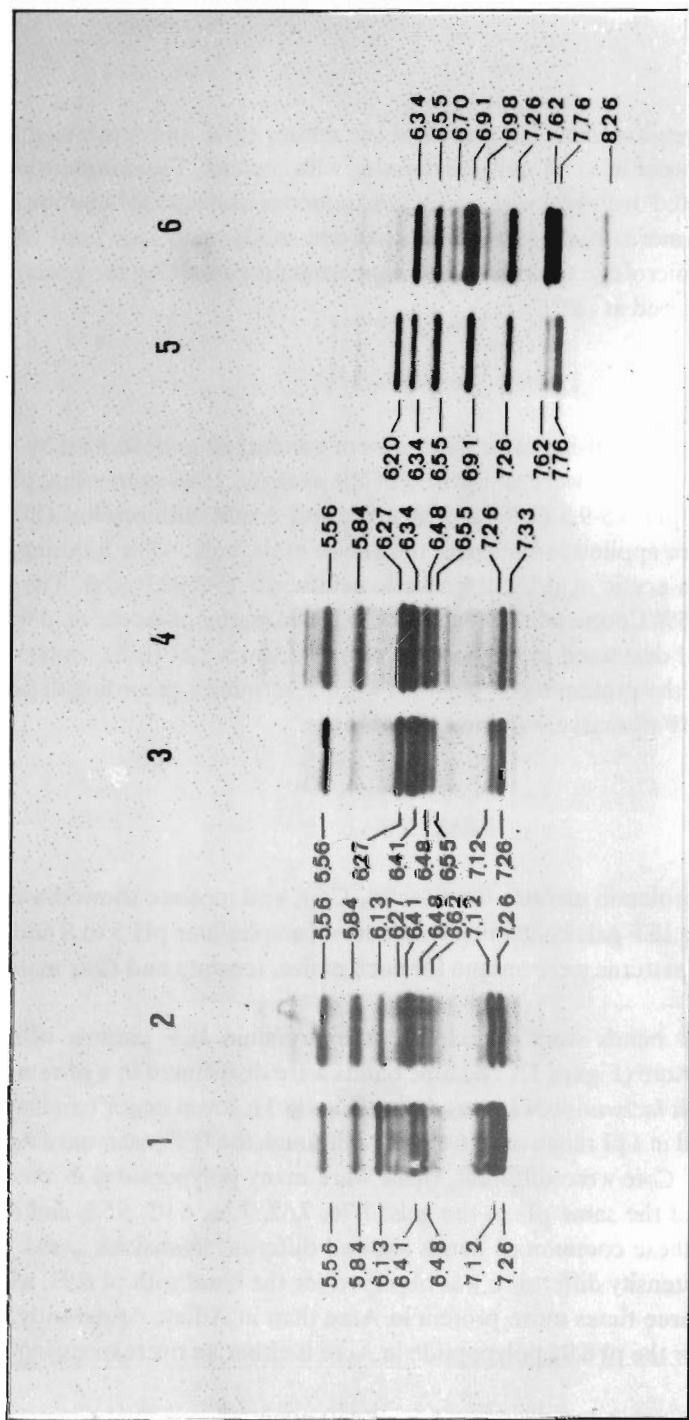


Figure 1 - Isoelectric focusing analysis of the prolamins. Lane 1: *Zea luxurians*, Lane 2: *Zea mays mexicana* - Chalco Los Reyes. Lane 3: *Zea mays* L. L1038. Lane 4: *Zea mays* L. var. Maya. Lane 5: *Coix lacryma-jobi* L. var. Adlay. Lane 6: *Coix lacryma-jobi* L. var. Acra. The prolamins were extracted with a 55% aqueous isopropanol solution containing 2% 2-mercaptoethanol. After focusing, the gel was stained with 0.5% Coomassie Brilliant Blue R.250 in methanol/water/acetic acid (20:70:10, v/v/v).

compared with the zein IEF pattern of the maize variety Maya which had eight major bands, distributed in a pI range of 7.33 to 5.56. It was observed that the maize IEF polypeptides were more grouped in some pI ranges than the IEF polypeptides of *Coix*. The two most basic zein bands with pIs 7.26 and 7.12 in the inbred line L1038 and pIs 7.33 and 7.26 in Maya were found 25 mm from the cathode of the gel. Following those bands, a group of four major bands was identified between 35 and 40 mm from the cathode. The pI of three out of four major grouped bands was the same for Maya and L1038. The pIs of those three bands were 6.55, 6.48, and 6.27. The band that differed was pI 6.35 in Maya and 6.41 in L1038. The most acidic bands with pIs 5.84 and 5.56 found in the IEF pattern of Maya were also found in the IEF pattern of L1038. The only difference was that the band with pI 5.84 had a medium intensity in L1038. Thus, this band could not be considered part of the seven major bands that best characterize the L1038 IEF profile. There was a similarity between the IEF pattern observed for Maya and L1038 and the one observed for the teosintes. The similarity was more evident for the teosinte *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes). There were nine major bands in this teosinte, distributed in the same pI range of 7.26 to 5.56 as the L1038 IEF bands (Figure 1). The four bands between 35 and 40 mm from the cathode of the gels in Maya and L1038 were also observed in *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes). Among those four bands, the ones with pI 6.48 and 6.27 were found in Maya and L1038, the band with pI 6.41 was found in L1038, and the one with pI 6.62 was a typical *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes) band. The two most acidic bands with pI 5.84 and 5.56 found in *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes) and previously identified in Maya and L1038 were also found in the teosinte *Zea mays* L. ssp. *luxurians*. Among the prolamin IEF profiles analysed, that of *Zea mays* L. ssp. *luxurians* seemed to be the most complex (Figure 1). In *Zea mays* L. ssp. *luxurians*, seven major bands were identified in the same pI range of 7.26 to 5.56 as the major bands of L1038 and *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes).

There were some bands that best characterized the maize, teosinte, and *Coix* strains. To determine how representative those bands were, other maize inbred lines and teosinte, and *Coix* strains had their prolamin extracted and submitted to IEF analysis. In that way, the zein IEF profile of Maya and L1038 was compared with the ones from the maize inbred lines L126-3, L107-1, and L75-3. It was observed that, besides Maya and L1038, the other maize inbred lines also have a distinct and heterogeneous zein IEF profile (Figure 2). The most basic IEF band observed among the maize IEF profiles was in the inbred line L107-1 at 18 mm from the cathode of the gel. The most acidic band was found in the inbred line L75-3, 58 mm from the cathode. Although the different maize genotypes have distinct zein profiles, there were certain characteristics that they shared, such as the group of four bands between 35 and 40 mm from the cathode of the gel, with pI between 6.55 and 6.27. Another characteristic shared by the maize genotypes was a band with pI 5.56.

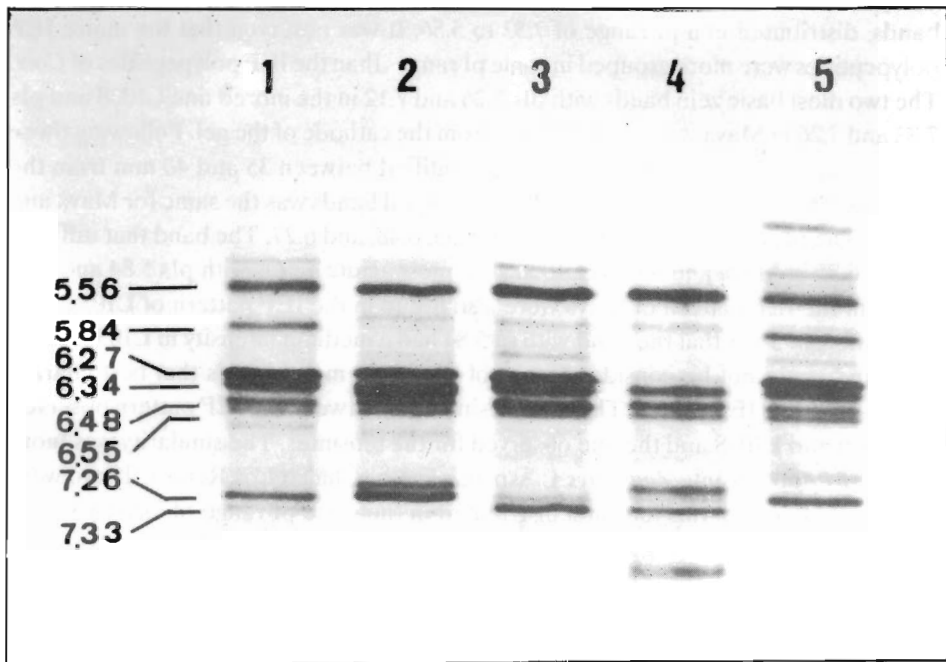


Figure 2 - Isoelectric focusing of maize prolamins. Lane 1: *Zea mays* L. var. Maya. Lane 2: *Zea mays* L. L1038. Lane 3: *Zea mays* L. L126-5. Lane 4: *Zea mays* L. L107-1. Lane 5: *Zea mays* L. L75-3.

There was a greater complexity in the prolamins IEF profiles of the teosintes than in the maize genotypes (Figure 3). The number of major and minor bands was greater in the teosintes IEF patterns. The most basic band observed among the teosintes was found in *Zea mays* L. ssp. *mexicana* (Batan) at 13 mm from the cathode of the gel. At 62 mm from the cathode, a well-defined acidic band was identified, especially intense in the teosintes *Zea mays* L. ssp. *mexicana* (Central Plateau), *Zea mays* L. ssp. *mexicana* (Balsas), and *Zea perennis*. The IEF pattern of the teosintes showed certain similarities with the IEF patterns of the maize genotypes, such as: (1) the band with pI 5.56, found in the IEF pattern of all maize genotypes analysed, was also found in all the teosintes; (2) the group of bands distributed in the pI range of 6.55 to 6.27 in the maize genotypes was also identified in the teosintes *Zea mays* L. ssp. *mexicana* (Chalco Los Reyes), *Zea mays* L. ssp. *mexicana* (Batan), and *Zea mays* L. ssp. *mexicana* (Balsas). The other three teosintes analysed also showed bands in that pI range, but the four-band grouping, a characteristic of the maize and some teosintes was less evident.

Five *Coix* strains were analysed and compared by prolamins IEF profile (Figure 4). No grouping of bands, as observed for the maize and teosinte genotypes, was detected in the *Coix* prolamins profiles. Another characteristic observed among

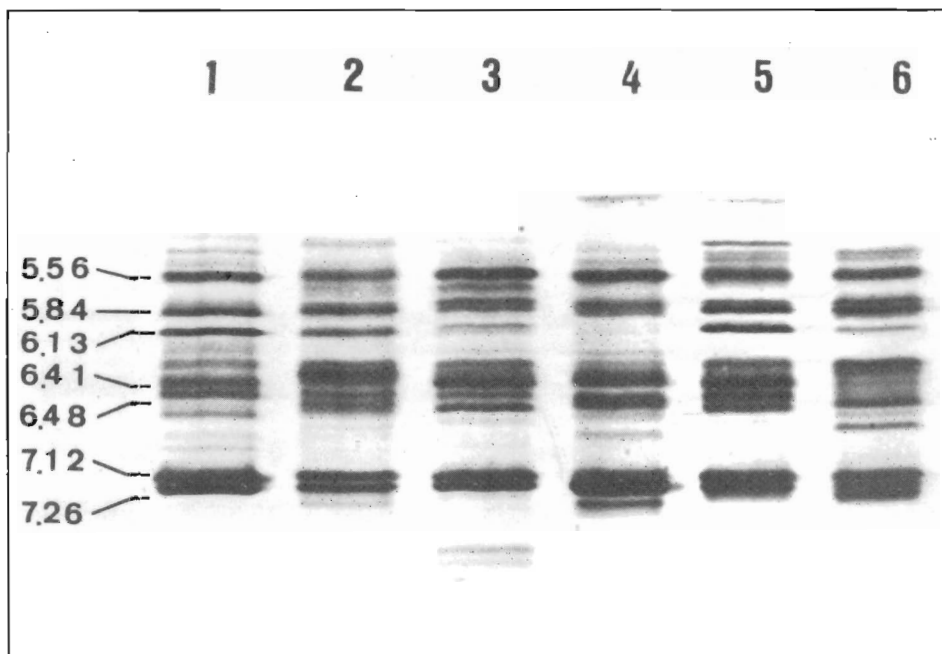


Figure 3 - Isoelectric focusing of teosinte prolamins. Lane 1: *Zea luxurians*. Lane 2: *Zea mays mexicana* - Chalco Los Reyes. Lane 3: *Zea mays mexicana* - Batan. Lane 4: *Zea perennis*. Lane 5: *Zea mays parviglumis* Balsas. Lane 6: *Zea mays mexicana* - Central Plateau.

the *Coix* genotypes was the similarity detected for the IEF profiles of that cereal. That is, there was a great homology between the IEF pattern of *Coix* Acre and Tailandia, and the IEF pattern of *Coix* Rosario and Faxina can be distinguished only by a few bands of low intensity. Most of the major bands found in the IEF pattern of Adlay were also identified in the IEF pattern of the other *Coix* genotypes analysed. The pIs of those bands were 7.76, 7.62, 7.26, 6.91, 6.55, and 6.34. The band with pI 6.91 had the same intensity in *Coix* Acre and Tailandia. The band at the most basic pI observed among the *Coix* genotypes was at 13 mm from the cathode of the gel in Acre and Tailandia strains. The most acidic band was found at 52 mm from the cathode of the gel in Adlay. There was evidence of more acidic bands in the *Coix* IEF profiles than the one at 52 mm from the cathode. However, because those bands were fuzzy, they were not taken into consideration.

DISCUSSION

The prolamins are the major storage proteins of maize, *Coix* and teosinte endosperm. When the maize prolamin, zein, is submitted to isoelectric focusing, a complex band pattern representing the α -zein is observed, indicating that the protein

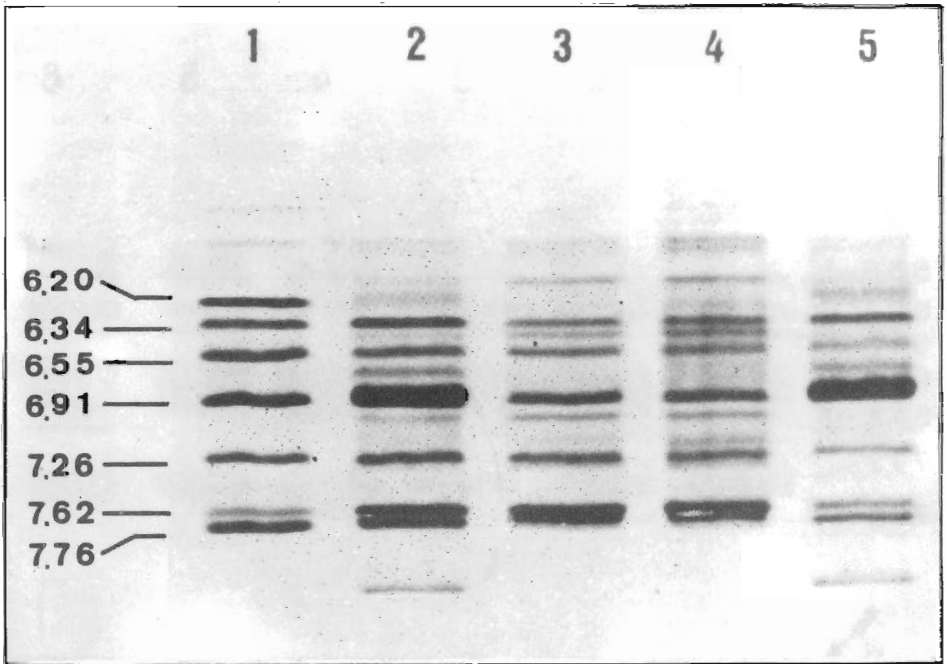


Figure 4 - Isoelectric focusing of *Coix* prolamins. Lane 1: *Coix lacryma-jobi* L. var. Adlay. Lane 2: *Coix lacryma-jobi* L. var. Acre. Lane 3: *Coix lacryma-jobi* L. var. Faxina. Lane 4: *Coix lacryma-jobi* L. var. Rosário. Lane 5: *Coix lacryma-jobi* L. var. Tailandia.

polypeptides differ in their net charge (Gianazza *et al.*, 1976). The same inference can be made for the prolamin polypeptides of *Coix* and teosinte, which showed extensive charge heterogeneity in the IEF gels. It has been suggested for maize that the charge heterogeneity observed in the IEF gels is due to differences in the structural genes that code for zeins (Gianazza *et al.*, 1977; Larkins *et al.*, 1979; Soave *et al.*, 1981). Based on the IEF profile variability of teosinte and *Coix* stocks analysed, it can be assumed that there should be many structural genes that code for the teosinte and *Coix* prolamins. The zein genes seem to have arisen due to gene duplication from a common ancestor of maize, teosinte, and *Tripsacum* (Wilson and Larkins, 1984). Some IEF bands (e.g. pI 5.56) were found in the IEF pattern of all the maize and teosinte genotypes analysed and might represent genes that were conserved as part of the maize and teosinte genome. Another characteristic observed in the maize and teosinte IEF patterns was the grouping of bands distributed in the pI range of 6.55 to 6.27.

Even though *Coix*, as well as maize and teosinte, belongs to the tribe *Andropogoneae*, the IEF pattern of the *Coix* genotypes analysed seems to have little homology with the IEF pattern of maize and teosinte. However, more experiments should be performed before any decisive evolutionary conclusions can be made. It has been suggested for maize that each IEF polypeptide represents a structural gene

(Soave *et al.*, 1981). If that is so, the gene that codes for the pI 6.91 band in *Coix* Acre and Tailandia must have gone through a duplication since the intensity of that band was much stronger in Acre and Tailandia than in the other *Coix* genotypes analysed. The similarities observed in the IEF pattern of *Coix* Acre and Tailandia and Rosário and Faxina suggest that there was a lack of genetic variability among those strains.

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RESUMO

As prolaminas de milho, teosinte e *Coix* foram extraídas e analisadas através de focalização isoeétrica. Cada genótipo de milho, teosinte e *Coix* analisado apresentou um padrão próprio de prolamina nos géis. Como previamente constatado para o milho e o teosinte, a heterogeneidade de carga observada no padrão de prolamina do *Coix* sugere que múltiplos genes codificam estas proteínas. Dentre as bandas de prolamina que caracterizam os genótipos de milho, pode ser mencionado um grupo de quatro bandas com pI entre 6,55 e 6,27 e uma banda com pI 5,56 encontradas em todos os genótipos de milho analisados. Alguns dos genótipos de teosinte analisados também apresentaram bandas distribuídas na faixa de pI de 6,55 a 6,27. A banda com pI 5,56 foi observada em todos os teosintes analisados. Com base nas homologias observadas, pode-se inferir que os padrões de focalização isoeétrica dos genótipos de milho apresentaram maior homologia com os padrões de focalização isoeétrica dos teosintes do que com aqueles observados para os genótipos de *Coix*.

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