Plant Science, 83 (1992) 169–180
Elsevier Scientific Publishers Ireland Ltd.

Synthesis and deposition of coixin in seeds of *Coix lacryma-jobi*

Maria Luisa Natividade Targon[a], Laura M.M. Ottoboni[a], Adilson Leite[a], Dolores Ludevid[b], Pedro Puigdomenech[b] and Paulo Arruda[a,c]

[a]*Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, 13081, Campinas, SP (Brazil),* [b]*Departamento de Genética Molecular, Centro de Investigacion y Desarrolo, CSIC, Jorge Girona 18-26, E-08034 Barcelona (Spain) and* [c]*Departamento de Genética e Evoluçao, IB, Universidade Estadual de Campinas, 13081, Campinas, SP (Brazil)*

(Received October 8th, 1991; revision received January 2nd, 1992; accepted February 5th, 1992)

The synthesis and assembly of prolamins into protein bodies of *Coix lacryma-jobi* seeds were investigated. Coixins, the *Coix* prolamins, are grouped into two distinct classes namely α- and γ-coixins. Alpha-coixins are constituted by four size classes, while γ-coixins comprise only one molecular weight class. SDS-PAGE and western blot analysis of prolamins extracted from endosperm during seed development showed that α-coixins are synthesized at earlier developmental stages than γ-coixin. In vitro translation of polysomal RNA attached to protein bodies isolated from mid-maturation endosperm showed that these polyribosomes are highly enriched in coixin messages. Polysomal RNAs isolated from all developmental stages were electrophoresed and probed with cDNA clones representing α- and γ-coixins. The results confirmed the earlier expression of α-coixins and also demonstrated that coixin RNA accumulation in the endosperm occurs mainly at seed mid maturation. Protein bodies isolated from immature endosperm contained all coixin components as determined by SDS-PAGE and western blot analyses. Immunocytochemical analysis by electron and light microscopy revealed that the coixin components are spread all over the protein bodies. The protein bodies are localized in the starchy endosperm cells filling the spaces left by the starch granules. They are surrounded by continuous membranes and are larger than the protein bodies described for maize.

Key words: *Coix*; coixin; polysomal RNA; protein bodies; prolamin synthesis

Introduction

The major storage proteins in most cereals is a group of alcohol soluble proteins called prolamins [1–3]. The prolamins are synthesized in the endosperm during seed development by membrane bound polyribosomes and accumulate in storage organelles called protein bodies [4–7]. Information about prolamin gene expression can be obtained from the analyses of protein and mRNA accumulation. These analyses showed that zeins, the maize prolamins, are accumulated in the endosperm during seed development and that their synthesis is under strict tissue-specific control and is developmentally regulated [8].

Coix lacryma-jobi L. together with maize, tripsacum and sorghum belongs to the grass tribe Andropogoneae [9–11]. This cereal is native to South-East Asia [12]. It has been used as a food source for humans and livestock, in the production of alcoholic beverages and as a medicinal plant [13–15]. The seeds of *Coix lacryma-jobi* contain around 20% protein, the major constituent of which is a prolamin called coixin [16]. In previous reports we characterized the coixins of *Coix lacryma-jobi* L. var. Adlay endosperm [17,18]. These coixins, when extracted from *Coix* endosperm with solutions containing 70% ethanol or 55% isopropanol plus 2% 2-mercaptoethanol (2-ME), account for more than 70% of the total seed protein. Like other cereal prolamins, the coixin polypeptides contain very low levels of the

Correspondence to: Paulo Arruda, Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, 13081, Campinas, SP, Brasil.
Abbreviations: DTT, dithiothreitol; IEF, isoelectric focusing; 2-ME, 2-mercaptoethanol; p*I*, isoelectric point; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis.

0168-9452/92/$05.00 © 1992 Elsevier Scientific Publishers Ireland Ltd.
Printed and Published in Ireland

essential amino acids lysine and tryptophan [17]. Based on differential solubility, coixins can be grouped into two distinct classes: α- and γ-coixins. Alpha-coixin, the most abundant *Coix* prolamin class, represents 85% of total coixin. It is composed of four size classes of 27 kDa (C1), 25 kDa (C2), 17 kDa (C4) and 15 kDa (C5) and is responsible for the charge heterogeneity of polypeptides observed on IEF gels [17,19]. The amount of protein in C1, C2, C4 and C5 α-coixin, estimated by densitometry of SDS-PAGE gels, is 8%, 56%, 17% and 15%, respectively. The 22-kDa (C3) γ-coixin represents 15% of total coixin. We have demonstrated that coixins are coded by a multigenic family homologous to the zeins [18].

In this paper we report results from analyses of the synthesis and deposition of α- and γ-coixin proteins and the accumulation of specific coixin mRNAs during the development of *Coix lacryma-jobi* L. endosperms.

Material and Methods

Plant material

Seeds of *Coix lacryma-jobi* L. var. Adlay were obtained from the germplasm collection of the Genetics Department, University of Campinas. Because of the difficulty in conducting controlled pollinations to obtain seeds at specific developmental stages, five stages were chosen, based on the seed coat color and endosperm fresh and dry weights (Fig. 1). Seeds were harvested, frozen in liquid nitrogen and stored at −70°C.

Prolamin extraction, electrophoresis and western blot analysis

Seeds at each developmental stage were freeze dried and their endosperms, obtained by hand dissection of 100 seeds, ground to a flour and defatted with acetone. The prolamins were extracted and quantified as described in a previous report [17]. The prolamins were analyzed by SDS-PAGE in 5–20% gradient gels as described by Laemmli [20]. Western blot analyses were carried out essentially as described by Ottoboni et al. [17] and Leite et al. [18].

Protein bodies isolation

Protein bodies were isolated from developing *Coix* endosperms according to modifications of the methodology described by Larkins and Hurkman [6]. *Coix* endosperms were ground in a cold mortar in liquid nitrogen. After thawing, the tissues were homogenized in 5 volumes of buffer A (200 mM Tris–HCl (pH 8.5); 200 mM sucrose; 60 mM KCl; 50 mM $MgCl_2$) and centrifuged at 500 × *g* for 5 min. Two milliliters of the supernatant were layered on top of a discontinuous sucrose gradient consisting of 2 ml of 2 M sucrose; 3.5 ml of 1.5 M sucrose and 3.5 ml of 0.5 M

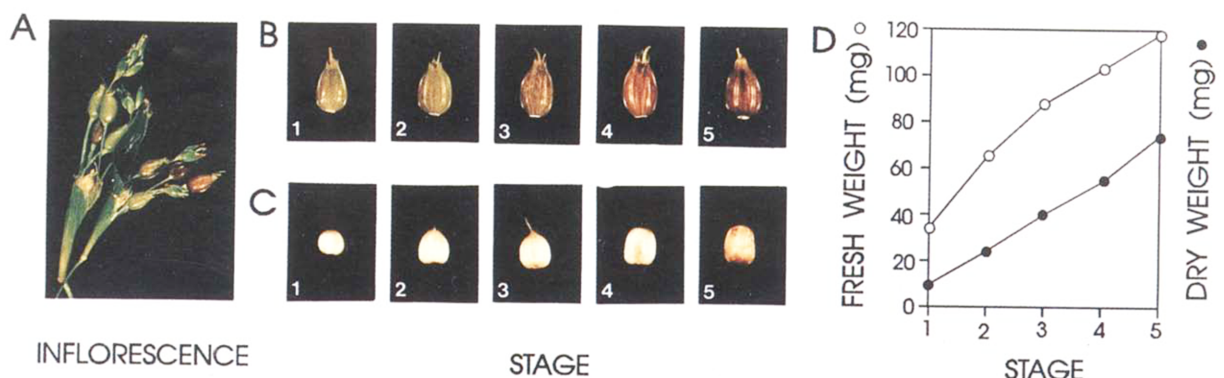


Fig. 1. Development of *Coix lacryma-jobi* L. var. Adlay seeds. (A) *Coix* branches showing flowers in the same tiller containing seeds at different developmental stages. (B) Seeds of *Coix* were classified into five different developmental stages based on the seed coat color and endosperm dry weight. (C) Endosperm development from stage 1 to 5. (D) Changes in the endosperm fresh (○) and dry (●) weight during development.

sucrose in buffer B (40 mM Tris–HCl, pH 8.5; 20 mM KCl; 10 mM $MgCl_2$). The gradient was centrifuged at 77 000 × *g* for 2 h at 4°C and the protein bodies localized between the 1.5- and 2-M sucrose layer were collected, diluted 5 times with buffer A without sucrose and then pelleted by centrifugation at 50 000 × *g* for 1 h at 4°C. The pellet was resuspended in TE (10 mM Tris–HCl, pH 8.0; 1 mM EDTA) and stored at −20°C. For SDS-PAGE analysis, protein bodies were lyophilized and the pellet was resuspended in 55% isopropanol plus 2% 2-ME.

Isolation of RNA in vitro translation and Northern blot analysis

Total RNA was isolated from the developing endosperms according to the methodology described by Naito et al. [21]. Quantification of total RNA at each endosperm developmental stage was carried out as described by Maniatis et al. [22].

Following protein body isolation, polysomal RNAs were purified from membrane bound polyribosomes by standard procedures [23]. Quantification of polysomal RNA at each stage was carried out as described by Maniatis et al. [22].

A sample of polysomal RNA taken from stage 3 endosperm was translated in vitro in a wheat germ cell-free system (Amersham International, Bucks, UK) according to the manufacturer's instructions with [^{35}S]methionine as radioactive protein precursor. Optimal concentration of K^+ was 150 mM. The products of the in vitro translation were analyzed by 12.5% SDS-PAGE and fluorography.

Samples of polysomal RNA were separated by electrophoresis in 1.2% agarose gels containing 6% formaldehyde and then transferred to Hybond-N membranes (Amersham) according to Maniatis et al. [22]. The membranes were hybridized with radiolabelled α- and γ-coixin cDNAs clones pBCX 27.10 and pBCX 22.3 (Leite et al., unpublished results) under conditions described in Leite et al. [18].

Immunocytochemistry

Stage 3 endosperms were cut longitudinally into 1-mm^3 sections and fixed for 1.5 h at room temperature in 4% (w/v) paraformaldehyde; 0.3% (v/v) glutaraldehyde in PBS (4 mM KH_2PO_4; 26 mM NaOH; 130 mM NaCl, pH 7.5) containing 2.5% (w/v) sucrose (PBS-S). Protein bodies were pelleted, washed with PBS-S for 5 min and fixed with 0.5% (v/v) glutaraldehyde in PBS-S for 30 min at room temperature. The endosperm and protein body preparations were neutralized with 0.2 M NH_4Cl in PBS twice for 10 min and washed with PBS three times for 10 min. They were then dehydrated at 4°C by successive treatments with increased ethanol solutions. Following dehydration, samples were infiltrated in LR White resin (London Resin Company Ltd., England) and used for the light and electron microscope studies.

Immature endosperm semi-thin sections (0.5–1.0 μm) were cut with ultramicrotome and collected onto glass slides treated in a solution containing 50 μg/ml poly-D-lysine hydrobromide in 10 mM Tris–HCl (pH 8.0). Sections were incubated for 10 min at room temperature with 10% (v/v) methanol; 3% (v/v) H_2O_2 in PBS. After incubation, sections were washed twice for 10 min in PBS and once for 10 min in PBS containing 0.1% (v/v) Tween-20 (PBS-T). The PBS-T was removed and the sections were saturated with solution I (PBS-T; 0.1% bovine serum albumin) for 2 h at room temperature. Following saturation, sections were incubated overnight at 4°C with anti-C2-α- and anti-γ-coixin antisera [17] diluted 1:100 in solution I. Preimmune serum at a similar dilution was used as a control. Slides were then washed three times for 10 min in PBS and the sections were incubated for 1 h at room temperature with Protein-*A*-conjugated to 10-nm gold particles (Sigma-Chemical Co., St. Louis, MO) diluted 1:30 in solution I. Slides were washed three times for 10 min in PBS, three times for 5 min in distilled water and then dried. Silver enhancement of the colloidal gold was done with IntenSE M (Jansen Life Science Products, Olen, Belgium) for 10 min according to the manufacturer's instructions. Sections were then observed in a Zeiss photomicroscope (Germany).

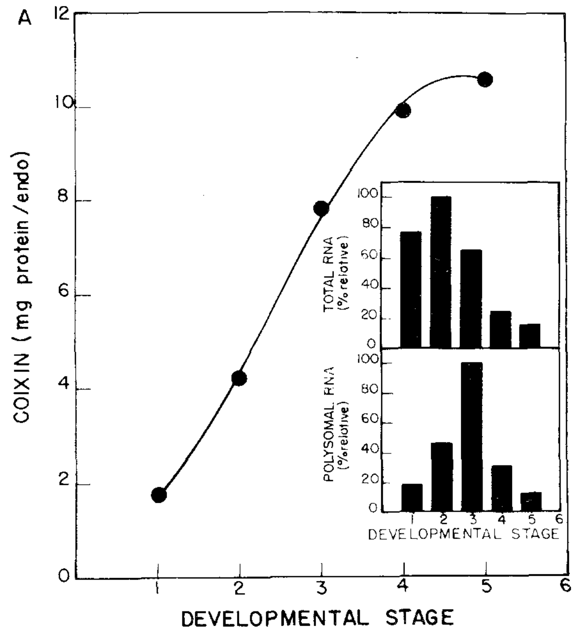
Protein body ultra-thin sections (70–80 nm) were cut with an ultramicrotome and collected on copper grids. The immunocytochemical staining was carried out with minor modifications of the

procedure described by Ludevid et al. [24]. The grids were washed twice for 5 min in PBS and the sections were saturated for 2 h at room temperature with solution I. Sections were then incubated overnight at 4°C with anti-C1, anti-C2-, anti-C4-, anti-C5-α-coixins antisera and anti-γ-coixin antiserum diluted 1:200 in solution I. Preimmune serum at a similar dilution was used as control. Following incubation, the grids were washed three times for 10 min in PBS and the sections were incubated for 1 h at room temperature with protein-A conjugated to 20-nm gold particles diluted 1:30 in solution I. Grids were washed three times for 5 min in PBS-T containing 0.5 M NaCl, three times for 5 min in PBS and then, thoroughly rinsed in PBS and distilled water. After staining for 30 min in 2% uranyl acetate and post staining for 10 min in Reynold's lead citrate, sections were observed in a Phillips, EM 301 electron microscope (Eidhoven, the Netherlands).

Results

Development of Coix seeds

The seeds of *Coix lacryma-jobi* L. var. Adlay are produced in apical branches of the tillering plant. Each branch contains 6–9 flowers, each of which has a seed coat inside of which the seed develops. The opening of each flower varies widely in the same branch and between branches in the same tiller (Fig. 1A). The seed coat does not change considerably in size and shape during seed development, but its color changes from green to dark brown (Fig. 1B). The change of the seed coat color can be used as an indicator of seed development, since it correlates very well with seed size (Fig. 1C) and dry weight (Fig. 1D). The period from the beginning of development to physiological maturity is around 35 days. Based on the seed coat color, we divided *Coix* seed development into five stages (Fig. 1B). Stage 1 represents the beginning of seed development, stage 2 an intermediary point between the beginning of development to middle maturation, stage 3 the mid maturation stage representing the most active stage in terms of protein synthesis and expression of coixin genes, stage 4 an intermediary point between middle maturation and physiological maturity and stage 5 the physiological mature seed.



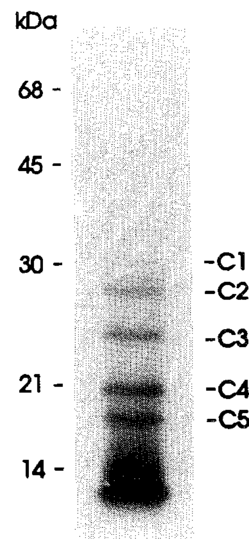


Fig. 2. (A) Comparison of protein and RNA accumulation during the development of *Coix* endosperm. Coixins, total and polysomal RNAs were extracted from endosperm at developmental stages 1–5. The levels of total RNA corresponded to 921.3, 1196.1, 526.4, 298.0 and 197.4 μg/g endosperm and the levels of polysomal RNA corresponded to 9.3, 12.1, 72.3, 27.5 and 10.2 μg/g endosperm. (B) Fluorography of the in vitro translation products of mRNA isolated from stage 3 endosperm separated by SDS-PAGE. Positions of protein standards of known molecular weights are indicated on the left and positions of C1, C2, C3, C4 and C5 pre-coixins are indicated on the right.

Protein and RNA accumulation during endosperm development

Total coixins extracted from endosperms at developmental stages 1–5 are presented in Fig. 2A. Coixins start to accumulate at stage 1 and reach maximum levels at stages 4 and 5. The amount of total coixin at the physiological maturity represents 70% of the total protein of *Coix* endosperm. The levels of total RNA were maximum at initial stages of seed development and then declined gradually towards seed maturity showing only small amounts in mature endosperm (Fig. 2A). The level of polysomal RNAs purified from isolated protein bodies was minimum at stage 1, reached maximum levels at stage 3 and then declined towards seed maturity (Fig.2A). As can be observed, the stage of maximum polysomal RNA accumulation corresponds to the stage of maximum rates of coixin accumulation.

Coixin precursors

Polysomal RNA isolated from stage 3 endosperm was in vitro translated and the products analyzed by SDS-PAGE and fluorography (Fig. 2B). The fluorograph presented the five coixins size classes, but with the exception of the γ-coixin, they were approximately 2 kDa larger than native coixins. The band below 14 kDa corresponds to the background produced by the translation kit and was also observed in the control (data not shown). Therefore this band could not be attributed to a methionine-rich coixin of lower molecular weight. In maize, it a 10-kDa methionine-rich zein has been observed, [25], but in *Coix* there is no evidence for such a protein.

Synthesis of coixins in developing endosperms

The time course synthesis of each coixin size class from stages 1–5 were analyzed by SDS-PAGE and Western blot (Fig. 3). With the development of the endosperm, a gradual increase in the amounts of all coixin classes was observed (Fig. 3A). At stage 1, C1, C4 and C5 α-coixins were barely detectable, while C2-α-coixin represented the most abundant class in this stage and throughout development. The γ-coixin could not be observed in the SDS-PAGE in the stage 1 endosperm. The five coixin classes were detected in stage 2. A similar staining intensity was observed

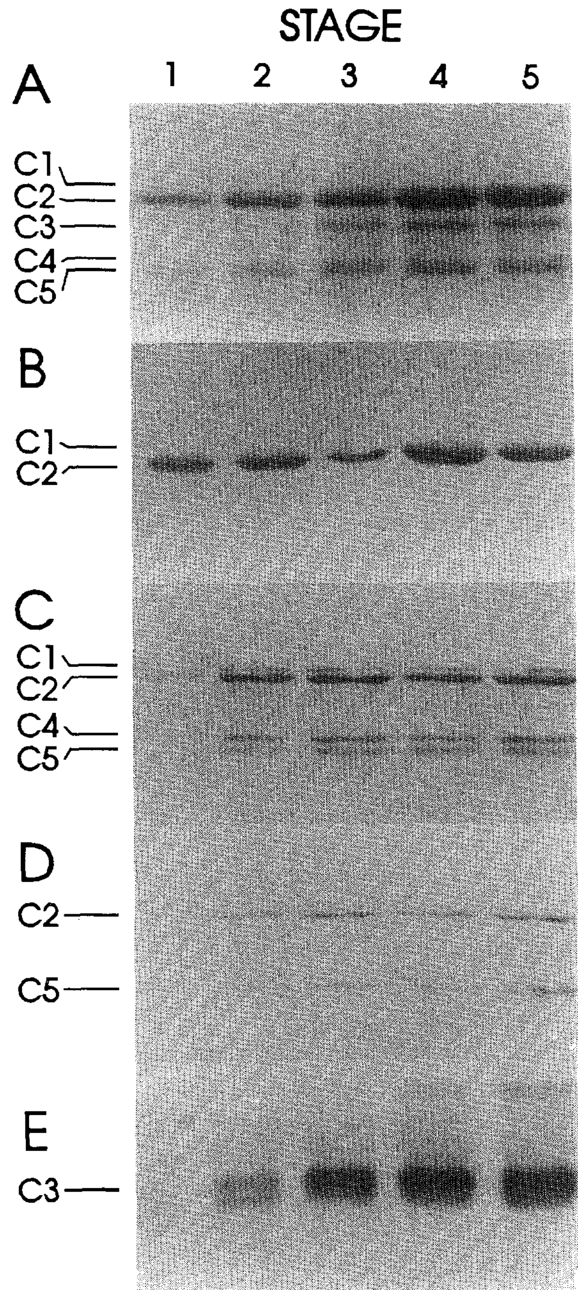


Fig. 3. (A) SDS-PAGE analysis of coixins extracted from endosperms at developmental stages 1–5. Coixins were stained with 0.25% Comassie Brilliant Blue R 250 in methanol/water/acetic acid (5:5:1 v/v). (B–E) Immunoblotting analysis of replicate SDS-PAGE gels from (A). Coixins were transferred to nitrocellulose membranes and incubated with anti-C1 + C2 (B), anti-C4 (C), anti-C5 (D) α-coixin and anti-γ-coixin (E) antisera.

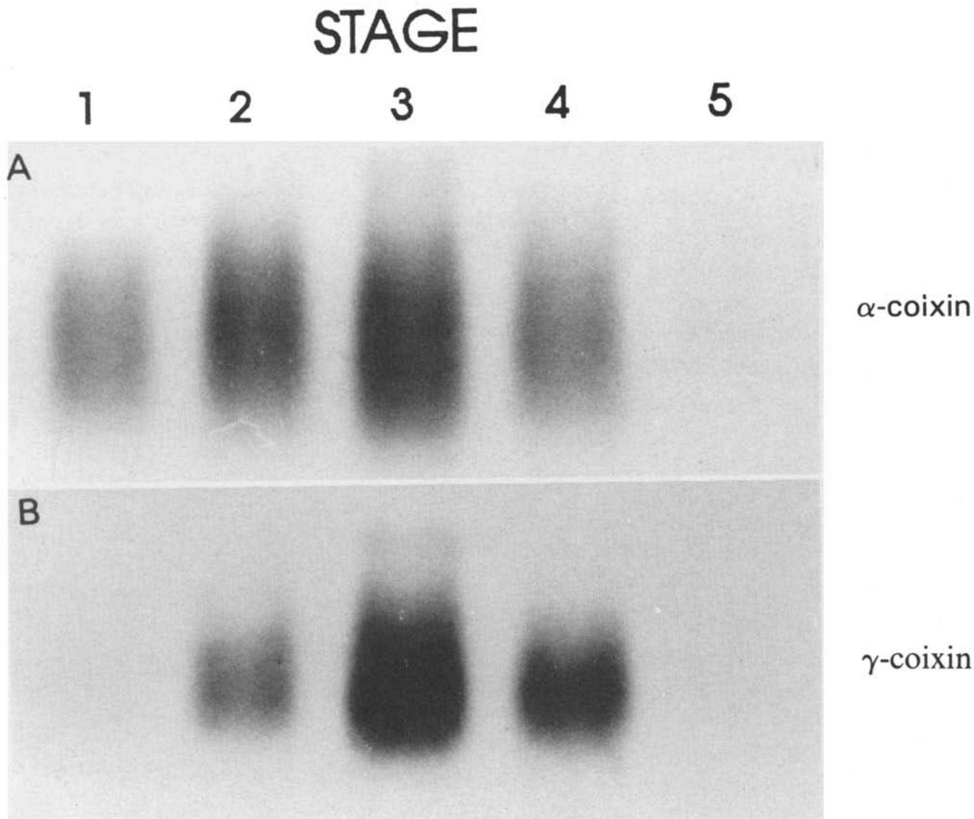


Fig. 4. Northern blot analysis of polysomal RNA isolated from endosperms at developmental stages 1–5. Equal volumes of RNA were fractioned on 1.2% agarose gels containing 6% formaldehyde. The polysomal RNA was transferred to Hybond N membranes and probed with the cDNA α-coixin clone pBCx27.10 (A) and with the cDNA γ-coixin clone pBCx 22.3 (B). Film exposure time was 1 h for RNA hybridized with pBCx 27.10 and 18 h for RNA hybridized with pBCx22.3.

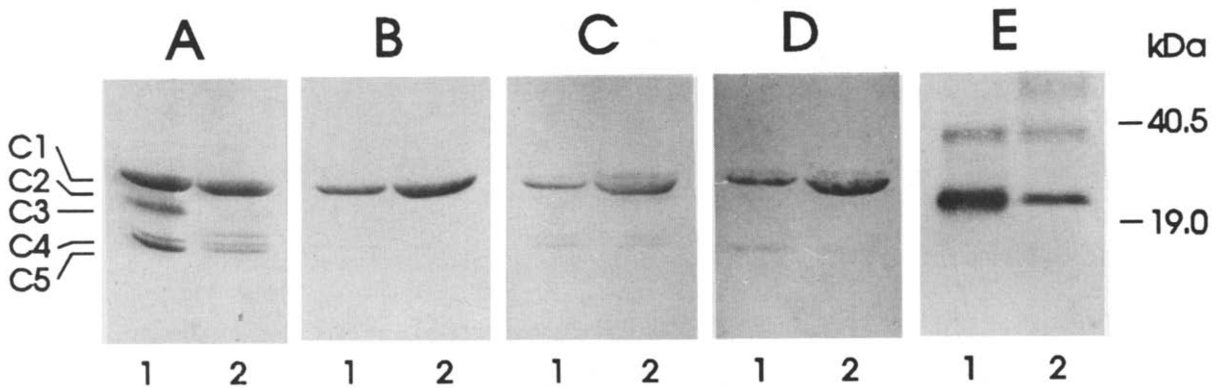


Fig. 5. (A) SDS-PAGE of coixins extracted from mature endosperm (lane 1) and from protein bodies (lane 2). (B–E) immunoblotting analysis of coixins of replicate gels from (A) using anti-C1 + C2 (B), anti-C4 (C), anti-C5 (D) α-coixin antisera, and anti-γ–coixin antiserum (E).

for C1, C4 and C5 α-coixin while γ-coixin was barely detected. In stage 3 all coixin classes were detected and the coixin classes reached maximal levels of accumulation in stages 4 and 5. Replicate gels from Fig. 3A were immunobloted with the antisera raised against C1 + C2 [17] (Fig. 3B), C4 (Fig. 3C) and C5 [18] (Fig. 3D) α-coixin classes and against the γ-coixin (Fig. 5E). This is particularly useful to detect the presence of γ-coixin since immunoblots of this protein are more sensitive than Coomassie blue stained gels [17]. The immunoblots confirmed the results obtained in the SDS-PAGE analysis, showing that α-coixins are synthesized in earlier stages than γ-coixin.

To investigate if the differential time course of α- and γ-coixin synthesis corresponds to differences in the timing of coixin gene expression, polysomal RNAs from each endosperm develop-

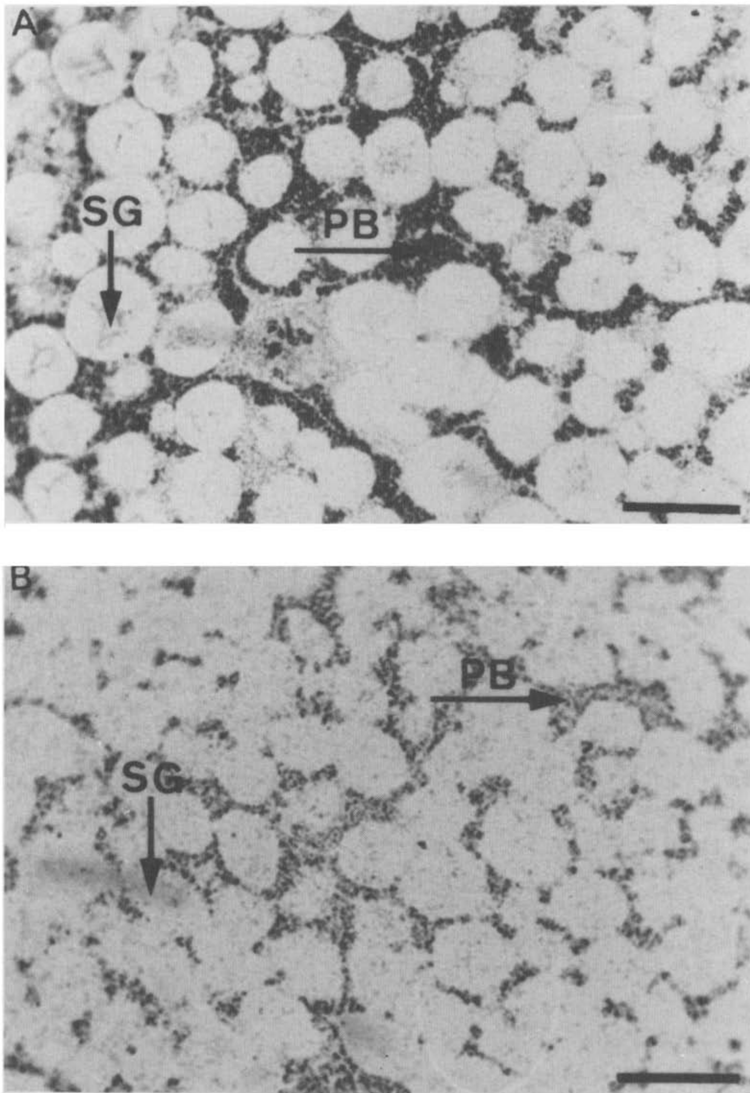


Fig. 6. Semi-thin sections of immature *Coix* endosperm included in LR White resin and incubated with anti-α- and γ-coixin antisera. Abbreviations used: SG, starch granules; PB, protein bodies. Bar = 5 μm. (A) Section incubated with anti-C2-α-coixin antiserum followed by Protein-A conjugated to 10-nm gold particles and treated with a silver enhancement mixture. (B) as (A), but with anti-γ-coixin antiserum.

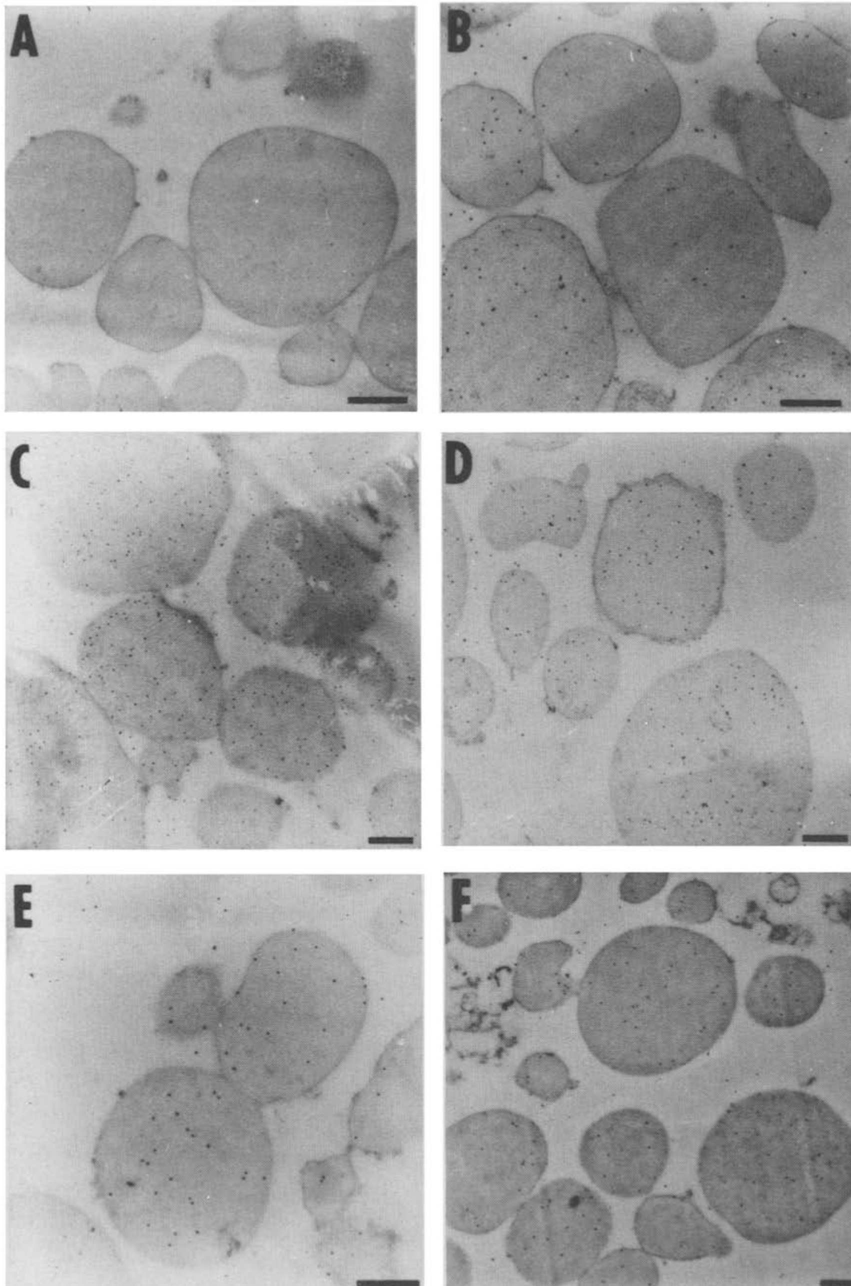


Fig. 7. Immunocytochemical analysis of protein bodies isolated from stage 3 *Coix* endosperm. Protein bodies ultra-thin sections were incubated with preimmune (A), antiserum; (B), anti-C1-; (C), anti-C2-; (D), anti-C4-; and (E), anti-C5-α-coixins antisera and (F), anti-γ-coixin antiserum followed by Protein-A conjugated to 20-nm gold particles. Bar = 0.5 μm.

mental stage were electrophoresed on 1.2% agarose gels, transferred to nylon membranes and hybridized to α-coixin cDNA clone pBCx27.10 and to γ-coixin cDNA clone pBCx22.3 (Fig. 4). The α-coixin messages began to accumulate in stage 1, reached their maximum level at stage 3 and then declined toward seed maturity, with non-detectable levels at stage 5 (Fig. 4A). The γ-coixin messages started to accumulate only in the stage 2 endosperm, reached a maximum level at stage 3 and then decreased towards seed maturity (Fig. 4B).

Deposition of coixins

The SDS-PAGE pattern of coixins extracted from protein bodies isolated from stage 3 endosperm (Fig. 5A, lane 2) revealed that all classes were present in these organelles and showed an extra-band with apparent molecular weight of 19 kDa, that was not present in the coixins extracted from mature endosperms (Fig. 5A, lane 1). To confirm this analysis, coixins from replicate gels from Fig. 5A were transferred to nitrocellulose membranes and incubated with antisera raised against C1 + C2 (Fig. 5B), C4 (Fig. 5C), C5 (Fig. 5D) α-coixins and against γ-coixin (Fig. 5E). The results of the immunoblots confirmed the SDS-PAGE analysis indicating that α- and γ-coixins are deposited in the protein bodies. None of the coixin antisera cross-reacted with the extra band of 19 kDa present in the SDS-PAGE of protein bodies.

Endosperm immunocytochemical staining

The deposition of coixins in the endosperm cells was analyzed by immunocytochemical staining and light microscopy. Sections incubated with anti-C2α-coixin (Fig. 6A) and with anti-γ-coixins (Fig. 6B) antisera followed by immunogold labelling showed that the protein bodies were embedded in the endosperm cells between the starch granules. The intensity of the immunostaining was greater for α- than for γ-coixin, probably due to the highest amount of α-coixins in the endosperm cells.

Immunocytolocalization of coixins into protein bodies

Ultra-thin sections of protein bodies isolated from stage 3 endosperm were immunostained with antisera raised against C1, C2, C4, C5 α- and against γ-coixins (Fig. 7). The samples stained with preimmune antiserum revealed the absence of non-specific labelling (Fig. 7A). Sections incubated with anti-C1, C2, C4, C5 α- and with γ-coixins (Fig. 7B–7F, respectively) showed that the five coixin classes were spread all over the protein bodies. Variation in the labelling intensity is due to the differential proportion of each coixin classes. The protein bodies isolated from stage 3 endosperm presented a great variation in size, with diameters ranging from 0.2 to 3.7 μm.

Discussion

Coixins are the major storage proteins of *Coix* seeds and account for more than 70% of total seed protein. These proteins were detected exclusively in the endosperm tissue (data not shown), which indicates that the coixin gene is under tissue-specific control. As can be observed in Fig. 2A, the coixin accumulation showed sigmoidal kinetics, following endosperm dry weight gain. The coixins and their specific mRNAs began to accumulate in early developmental stages, with a clear accumulation of polysomal mRNA at the stages with high rates of coixin synthesis. As coixin is synthesized and stored, the polysomal mRNA content decreases until it is non-detectable in the mature seeds (Fig. 2A).

The in vitro translation of polysomal RNAs isolated from protein bodies showed basically the five coixin classes as products (Fig. 2B). The apparent molecular weights of 30, 17, 22, 19 and 17 kDa indicated that the translation products of α-coixins were approximately 2 kDa larger than the native proteins. This result suggests that the α-coixins, like α-zeins [6,26] and other cereal prolamins [27,28], are synthesized as precursors. In contrast to α-coixins, no alteration of apparent molecular weight was observed for the translation product corresponding to γ-coixin. Similar results were reported for γ-zein [29]. Since the products of the in vitro translation showed mainly the five coixin classes in the SDS gels, it can be assumed that the polysomal RNA quantified in Fig. 2A represents coixin mRNAs.

The time course of specific coixin mRNA production and protein synthesis is not concomitant for all coixin classes. The SDS-PAGE and western blot analysis showed that the α-coixins C1, C2, C4, C5 were synthesized at earlier developmental stages than γ-coixin (Fig. 3). This was well correlated with the finding that α-coixin mRNA is present in the developing endosperm by stage 1, whereas γ-coixin mRNA appeared mainly in stage 2. The α- and γ-coixin mRNA reached maximal levels of accumulation at stage 3, which coincides with the stage of maximal protein accumulation. This correlation was also observed for storage proteins of oat [30], maize [31–33] and barley [28].

The results of SDS-PAGE and western blot analysis of coixins extracted from mature endosperm and from protein bodies showed that the five coixin classes are present in these organelles (Fig. 5). As was observed for maize and sorghum [4,6,34], the protein bodies of *Coix* endosperm are also formed as prolamin deposits.

The immunocytochemical analysis showed that the protein bodies are embedded between the starch granules, as observed in other cereals [35]. The protein bodies from *Coix* endosperm showed a great size variation, probably due to their localization in different endosperm cell layers, as suggested by Lending and Larkins [36] in maize.

Based on earlier findings of Ludevid et al. [24] and Lending et al. [37], a model for zein deposition into the protein bodies of developing maize endosperm was proposed by Lending and Larkins [36]. The authors showed that the γ- and β-zeins are initially deposited into the endoplasmic reticulum as dark-staining deposits around the peripherical layers, while α-zein was present at reduced levels or was absent. The subsequent accumulation of α-zeins was observed as light-staining deposits in the internal region of the protein bodies. As maturation occurs, α-zeins can be observed filling the central region of the protein bodies, surrounded by the β- and γ-zeins thin layer. However, this pattern of distribution varies substantially among different protein bodies. The immunostaining with anti-β-zein or anti-γ-zein antisera showed that these two protein classes present complex pattern of distribution, sometimes only in the periphery, sometimes spread all over the organelle and sometimes forming complex locule structures [37].

There was no indication of the localization of γ-coixin in the periphery and α-coixin in the internal regions of the *Coix* protein bodies as observed for maize. This could be attributed to the timing of protein deposition and packing of each prolamin class. Therefore in *Coix* the distribution of coixins could be more variable than for zeins in maize. Ludevid et al. [24] suggested that γ-zein has a structural function involved in the maintenance of the integrity of the maize protein body. The organelle dramatically changes its structure when protein bodies are isolated in the presence of 2-ME which solubilize γ-zein. We can not assume that γ-coixin has a similar function in *Coix* protein bodies. Other patterns of protein distribution have been observed also in rice where the different storage proteins can be observed in different kinds of protein bodies [38,39].

Acknowledgements

This work was financed by grants to P. Arunda from FINEP, PADCT, CABIO, CNPq/Proj. Integr. and FAPESP/PTE-90/3808–5. P. Arunda and A. Leite received research fellowship from CNPq. M.L.P.N. Targon was supported by Sementes Agroceres (S.A.) and received postgraduate fellowship from CAPES. L.M.M. Ottoboni received postgraduate fellowships from CAPES and FAPESP and a research fellowship from CNPq. The authors wish to express their gratitude to the coordination of the Servei de Microscopia Electronica de la Universitat de Barcelona, for the facilities offered in the electron microscopy studies. We thank Jose Andres Yunes for the artwork.

References

1 T.B. Osborne, Our present knowledge of plant proteins. Science, 28 (1908) 417–427.

2 B.J. Miflin and P.R. Shewry, The biology and biochemistry of cereal seed prolamins, in: Seed Protein Improvement in Cereals and Grain Legumes., IAEA, Vienna, Vol. 1, 1979, pp. 137–158.

3 B.A. Larkins, Seed storage proteins: characterization and

biosynthesis, in: P.K. Stumpf, E.E. Conn (Eds.), The Biochemistry of Plants, Vol. 6, Academic, New York, 1981, pp. 449–489.
4 U. Khoo and M.J. Wolf, Origin and development of protein granules in maize endosperm. Am. J. Bot., 57 (1970) 1042–1050.
5 B. Burr, and F.A. Burr, Zein synthesis in maize endosperm by polyribosomes attached to protein bodies. Proc. Natl. Acad. Sci. U.S.A., 73 (1976) 515–519.
6 B.A. Larkins and W.J. Hurkman, Synthesis and deposition of zein in protein bodies of maize endosperm. Plant Physiol., 62 (1978) 256–263.
7 J.C. Pernollet, The protein bodies of seeds: ultrastructure, biochemistry, biosynthesis and degradation. Phytochemistry, 17 (1978) 1473–1480.
8 M.D. Marks, J.S. Lindell and B.A. Larkins, Quantitative analysis of the accumulation of zein mRNA during maize endosperm development. J. Biol. Chem., 260 (1985) 16445–16450.
9 W.D. Clayton, The awnless species of Andropogoneae. Kew Bull., 28 (1973) 49–58.
10 W.D. Clayton, Notes on tribe Andropogoneae (Gramineae) Kew Bull., 35 (1983) 813–818.
11 S.K. Jain and D.K. Banerjee, Preliminary observations on the ethnobotany of the genus *Coix*. Econ. Bot., 28 (1974) 38–42.
12 R.K. Arora, Job's tears (*Coix lacryma-jobi*) — a minor food and fooder crop from North Eastern India. Econ. Bot., 31 (1977) 358–366.
13 R.V. Shaaffhausen, Adlay or Job's tears — a cereal of potentially greater economic importance. Econ. Bot., 6 (1952) 216–227.
14 H.F. Raimo and D.R. Leme, Contribuiçao para o estudo dos substitutos do farelo de trigo na alimentaçao das aves: I cereal Adlay. Bot. Ind. Anim., 11 (1950) 85–89.
15 P. Torres and A.O. Bergamin, O cereal do Adlay. Estudo de seu valor agricola e nutricional para aves. An. Esc. Super. Agric. Luiz de Queiroz, Univ. Sao Paulo, 8 (1951) 669–685.
16 J. Venkateswarlu and R.S.K. Chaganti, Job's Tears (*Coix lacryma-jobi* L.) ICAR Tech. Bull. (Agric.), 43–44 (1973) 1–54.
17 L.M.M. Ottoboni, A. Leite, M.L.P.N. Targon, A. Crozier and P. Arruda, Characterization of the storage protein in seed of *Coix lacryma-jobi* var. Adlay. J. Agric. Food Chem., 38 (1990a) 631–635.
18 A. Leite, L.M.M. Ottoboni, M.L.P.N. Targon, M.J. Silva, S.R. Turcinelli and P. Arruda, Phylogenetic relationship of zeins and coixins as determined by immunological cross-reactivity and Southern blot analysis. Plant Mol. Biol., 14 (1990) 734–751.
19 L.M.M. Ottoboni, A. Leite, M.L.P.N. Targon, M.J. Silva and P. Arruda, Heterogeneity of *Coix*, maize and teosinte prolamins detected by isoelectric focusing. Rev. Bras. Genet., 13 (1990b) 313–322.
20 U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227 (1970) 680–684.
21 S. Naito, P.H. Dubé and R.N. Beachy, Differential expression of conglycinin α- and β-subunit genes in transgenic plants. Plant Mol. Biol., 11 (1988) 109–123.
22 T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning : A Laboratory Manual. (1982) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
23 B. Mechler, Isolation of messenger RNA from membrane bound polysomes. Methods Enzymol., 152 (1987) 241–248.
24 M.D. Ludevid, M. Torrent, J.A. Martinez-Izquierdo, P. Puigdomenech and J. Palau, Subcellular localization of glutelin-2 in maize (*Zea mays* L.) endosperm. Plant Mol. Biol., 3 (1984) 227–234.
25 M.S. Benner, R.L. Phillips, J.W. Messing, Genetic analysis of methionine-rich storage protein accumulation in maize. Theor. Appl. Genet., 78 (1989) 761–767.
26 B. Burr, F.A. Burr, I. Rubenstein and M.N. Simon, Purification and translation of zein messenger RNA from maize endosperm protein bodies. Proc. Natl. Acad. Sci. U.S.A., 75 (1978) 696–700.
27 G.R. Donovan, J.W. Lee and T.J. Longhurst, Cell-free synthesis of wheat prolamins. Aust. J. Plant Physiol., 9 (1982) 59–68.
28 J.A. Matthews and B.J. Miflin, In vitro synthesis of barley storage proteins. Planta, 149 (1980) 262–268
29 M. Torrent, E. Poca, N. Campos, M.D. Ludevid and J. Palau, In maize, glutelin-2 and low molecular weight zeins are synthesized by membrane-bound polyribosomes and translocated into microsomal membranes. Plant Mol. Biol., 7 (1986) 393–403.
30 A.C. Brineagar and D.M. Paterson, Synthesis of oat globulin precursor. Analogy to legume 11S storage protein synthesis. Plant Physiol., 70 (1982) 1767–1769.
31 A. Viotti, E. Sala, R. Marotta, P. Alberi, C. Balducci and C. Soave, Genes and mRNAs coding for zein polypeptides in *Zea mays*. Eur. J. Biochem., 102 (1979) 211–222.
32 W.D. Park, E.D. Lewis and I. Rubenstein, Heterogeneity of zein mRNA and protein in maize. Plant Physiol., 65 (1980) 98–106.
33 L. Pérez-Grau, J. Cortadas, P. Puigdomenech and J. Palau, Accumulation and subcellular localization of glutelin-2 transcripts during maturation of maize endosperm. FEBS Lett., 202 (1986) 145–148.
34 J.R.N. Taylor, L. Schussler and N.v.d.W. Liebonberg, Protein body formation in the starchy endosperm of developing *Sorghum bicolor* (L.) Moench seeds. S. Afr. J. Bot., 51 (1985) 35–40.
35 J.C. Pernollet and J. Mosse, Structure and location of legume and cereal seed storage proteins, in: J. Daussant, J. Mosse and J. Vaughan (Eds)., Seed Proteins, Academic Press, London, 1983, pp. 155–191.
36 C.R. Lending and B.A. Larkins, Changes in the zein com-

position of protein bodies during maize endosperm development. Plant Cell, 1 (1989) 1011–1023.

37 C.R. Lending, R.S. Chesnut, K.L. Shaw and B.A. Larkins, Structure of maize protein bodies and immunocytochemical localization of zeins. Protoplasma, 143 (1988) 51–62.

38 D.R. Bechtel and B.O. Juliano, Formation of protein bodies in the starchy endosperm of rice (*Oryza sativa* L.): a reinvestigation. Ann. Bot., 45 (1980) 503–509.

39 K.J. Oparka and N. Harris, Rice protein body formation: all types are initiated by dilation of the endoplasmic reticulum. Planta, 154 (1982) 184–188.