



A new 2S albumin from *Jatropha curcas* L. seeds and assessment of its allergenic properties

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ABSTRACT

Significant effort has been made world-wide to boost biofuels with the expectation of a positive contribution to renewable fuel and greenhouse gas reduction. *Jatropha curcas* L. has proved to be an opportunistic crop in tropical areas, particularly in unfavorable environments. For this reason, analyses of toxicity and allergy caused by its seeds and pollen are necessary. A 12 kDa, allergenic 2S albumin, denoted Jat c 1, was isolated from Physic nut (*J. curcas*) seeds. Jat c 1 binds IgE attached to rat mast cells, inducing histamine release. It also showed strong cross-reactivity with the major allergens from castor bean, Ric c 1 and Ric c 3.

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1. Introduction

The physic nut is a small tree or large shrub which can reach a height of up to 5 m. The plant has 5–7 shallow lobed leaves, which are arranged alternately. The species is monoecious and flowers are unisexual; occasionally hermaphrodite flowers occur. The ripe fruits are yellow and the seeds are black with caruncle. The seeds are rich in unsaturated fatty acids. *J. curcas* is a drought-resistant species cultivated in the tropics [9].

J. curcas oil has been considered as a prospective feedstock for biodiesel production, particularly due to the possibility of cultivation in dry and marginal lands. However, for use in automobiles, *Jatropha* oil needs to be converted to biodiesel [19]. The oil content in *Jatropha* seeds is around 30–40% and it is potentially the most valuable end product, with properties such as low acidity, good oxidation stability compared to soybean oil, low viscosity compared to castor oil and better cold properties compared to palm oil. In addition, the viscosity, free fatty acids and density of the oil and the biodiesel are stable within the period of storage [25].

Once the seeds have been pressed to obtain the oil, the remaining seed meal can be used as feed in digesters and gasifiers to produce biogas for cooking and engines, or can be used as fertilizer. In Brazil, the use of *J. curcas* oil is increasing because it is thought to be less toxic than castor bean. However, it has a toxin (curcin) similar to ricin from castor bean that is capable of inhibiting protein synthesis [24], and phorbol esters [20] that are toxic and carcinogenic. Nothing is known about the presence of allergens in *J. curcas* seeds. Seeds and pollen in general present allergenic proteins with additional defense properties such as insect protease or amylase inhibitors or antifungal factors. Though protective for the plant, these anti-nutritional and toxic factors may have deleterious effects or even be toxic to animals and men [15].

IgE-mediated reactions are believed to be responsible for most induced allergic reactions of the immediate hyper sensibility type (Type 1), and the diagnosis relies on specific biological and clinical features. Such allergic reactions involve activation of effector cells, mainly mast cells and basophiles, leading to an inflammatory response and specific clinical manifestations [5].

The major allergens from castor bean are Ric c 1 and Ric c 3, which are proteins from the 2S albumin family [13,14,26]. Medical problems such as conjunctivitis, rhinitis and urticaria have been associated with the seed, as well as the pollen of castor bean [3,18,19,21]. In this study we purified and partially characterized the first known allergenic protein from *J. curcas* seeds. This protein

Abbreviations: PCA, passive cutaneous anaphylaxis; PEC, pyridylethyl-S-cysteine.

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presented characteristics similar to 2S albumins from other sources and it was able to cross-react with IgE against 2S albumin from castor bean, promoting rat mast cell degranulation *in vitro*. *In vivo*, the 2S albumin of *J. curcas* also cross-reacted with allergens from castor bean, as demonstrated by the PCA test.

2. Materials and methods

2.1. Plant material and 2S albumin purification

J. curcas seeds were obtained commercially from Mato Grosso do Sul/Brazil. Decorticated seeds (7.91 g) were finely ground with a pestle and mortar. Lipids were extracted using hexane 1:6 (*m/v*) overnight. Proteins from defatted powder (4.60 g) were extracted with 200 mM sodium phosphate buffer (pH 7.0) 1:5 (*m/v*) for 3 h. The extract was centrifuged in sealed containers for 20 min at $17,000 \times g$ and the upper fat layer was removed. Proteins in the supernatant were precipitated by 90% saturated ammonium sulfate solution at 4 °C overnight and centrifuged at $17,000 \times g$ for 20 min. The pellet obtained was pooled and suspended in distilled water. The suspension was heated for 15 min in a boiling water bath to inactivate the *J. curcas* toxin, curcin, which was subsequently removed by centrifugation at $1000 \times g$ for 20 min. The supernatant was freeze-dried, and the resulting powder was suspended in 10 mM sodium phosphate buffer pH 7.0, filtered through a 0.45 μm membrane and applied to a Sephadex G-50 medium column (1.5/27 cm) using the same buffer for elution. Fractions of 1 mL were collected at a flow rate of 1.0 mL/min and proteins were detected at 280 nm. The 2S albumin fraction was pooled and freeze-dried. The protein concentration was determined according to Bradford using ovalbumin as the standard [4].

2.2. Reverse-phase chromatography HPLC

The pool of 2S albumin was subjected to reverse-phase liquid chromatography on a C18 column (Sephacryl peptide C18 5 μm ST 4.6/250 from Pharmacia Biotech) using a Shimadzu apparatus. The chromatography was performed at a flow rate of 0.7 mL min⁻¹ using 0.1% TFA as solvent A and 80% acetonitrile containing 0.1% TFA as solvent B. The gradient was 0–80% of solvent B for 55 min. The elution profile was monitored by on-line measurement of the absorbance at 220 nm. Fractions containing the major peaks were dried in a speed Vac system. The UV spectrum was obtained using a photo-diode detector. The major protein was re-purified on a C18 column in the same conditions described above.

2.3. SDS-PAGE

Total protein extracts were resolved by SDS-PAGE 15%, as described by Laemmli [11].

2.4. Denaturation, reduction and pyridylethylation of the 2S protein

The 2S albumin (500 μg) was dissolved at 2.5 mg/mL in a solution containing 6 M guanidine chloride, 0.1 M Tris-HCl buffer, pH 8.3 with 1 mM EDTA for 2 h at 37 °C. Reduction and pyridylethylation were done as described by Allen [1]. The reaction mixture was submitted to C2/C18 column reverse-phase HPLC for small and large chain separation. Chromatography was performed at a flow rate of 0.5 mL min⁻¹ using 2% acetonitrile containing 0.065% trifluoroacetic acid (TFA) as solvent A and 80% acetonitrile containing 0.05% TFA as solvent B. The gradient was 0–80% of solvent B for 60 min. Proteins were detected by monitoring the absorbance at 220 nm. The UV spectrum was obtained using a photo-diode detector.

2.5. N-terminal amino acid sequences

The N-terminal partial amino acid sequences were obtained on a Shimadzu PPSQ-10 automated protein sequencer using the Edman degradation principle. The polypeptide sequences obtained were submitted to automatic alignment using a BLAST search system [2].

2.6. Tricine-SDS-PAGE

The small and large chains of the 2S albumin from *J. curcas* were resolved by tricine-SDS-PAGE [22].

3. Immunological characterization

3.1. Animals and antiserum

Isogenic female Balb/c mice were obtained from the animal house of the Universidade Federal Fluminense, Niteroi, RJ, and all experimental procedures were approved by the animal research ethics committee of this University. Mice were immunized by an intraperitoneal injection of 30 μL of saline containing 10 μg of F2H (a 2S albumin fraction from *J. curcas*) in the presence of an adjuvant mixture: 2.9 mg of Al_2OH_3 + 2.1 mg $\text{Mg}(\text{OH})_2$. After 28 days, the animals were given a booster with 5 μg of the antigen and 17 days later mice were anesthetized and bled by cardiac puncture. The sera were tested using the passive cutaneous anaphylaxis (PCA) test.

Female Lou-M adult rats weighing 150 g, reared at the animal house of the Universidade Federal Fluminense, Niteroi, RJ, were used throughout.

3.2. Passive cutaneous anaphylaxis

The PCA test was performed in rats according to the technique described by Braga and Mota [5] with use of a 72 h sensitization period for the IgE antibody. A shaved area of the dorsal region of two Lou-M rats was injected intradermally with 30 μL of mice serum anti-F2H from *J. curcas* diluted 1:40. After 72 h, PCA reactions attributable solely to antibodies of the IgE class were elicited by challenging the sensitized rats by intravenous injection of 500 μg of F2H or 2S albumin from castor bean in 0.5 mL saline mixed with 1% Evans blue dye. Saline (0.5 mL) was used as a negative control. Thirty minutes after the challenge, the rats were killed and the dorsal skin was removed and inverted to measure the area of pigment; the lesion diameters were measured and the area of the lesion was calculated in millimeters. Reactions were considered positive for spots larger than 5 mm in diameter.

3.3. Mast cell degranulation assay and histamine quantification

These methodologies were performed as described by Felix et al. [7] using rat antiserum against 2S albumin from castor seeds.

4. Results

4.1. A new 2S albumin from *J. curcas* seeds

The 2S *J. curcas* albumin fraction from *J. curcas* was isolated after saline extraction and chromatography on Sephadex G-50. Three fractions (F1–F3) were obtained after gel filtration chromatography (Fig. 1A). The elution profile was similar to that reported for the castor bean [23] and yellow mustard [28]. The region corresponding to 2S albumin (fraction F2) was submitted to reverse phase chromatography using a C18 column. A single peak of protein (F2H) was detected (Fig. 1B). The homogeneity of the

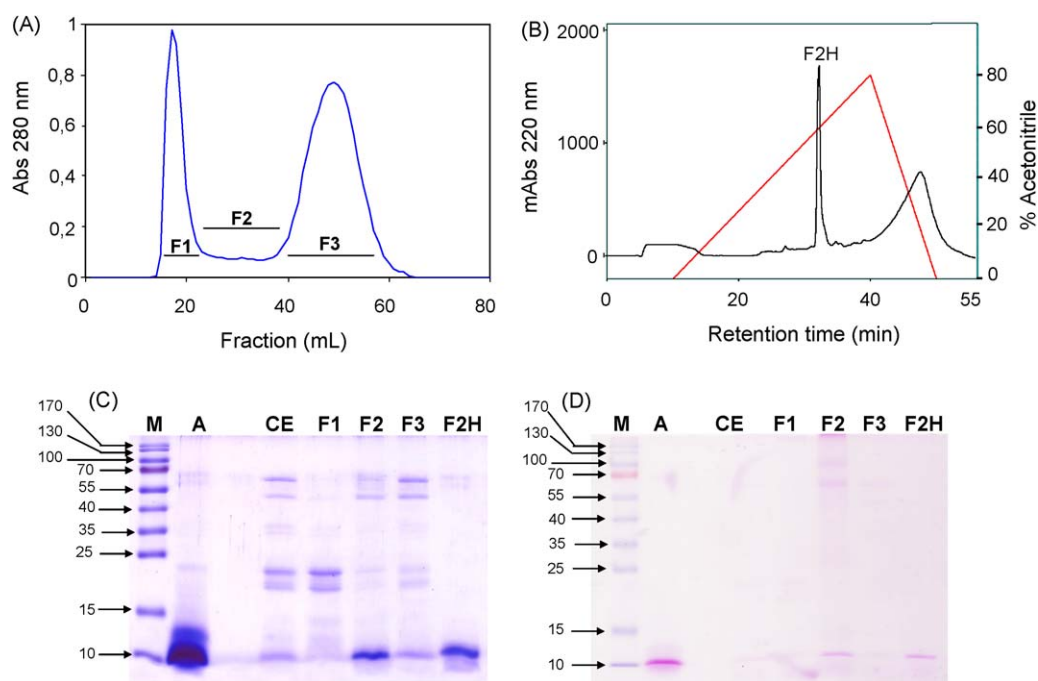


Fig. 1. Elution profiles of *J. curcas* protein extracts during purification of Jat c1 1: (A) gel filtration on Sephadex G-50 column; (B) separation of F2 fraction on a reverse phase C18 column. The oblique line represents the acetonitrile gradient; (C) SDS-PAGE 15% separation of *J. curcas* proteins from different steps of purification: M, broad range molecular marker; A – 2S albumin from castor bean; CE – *J. curcas* crude proteins extract; F1–F3, Sephadex G-50 peaks; F2H, fraction from C18 column. (D) A similar SDS-PAGE stained with Schiff's reagent. Protein load in each lane was 5 μ g, except lane A that present 20 μ g.

fractions obtained after different purification steps was analyzed by SDS-PAGE (Fig. 1C) and one major protein band was detected for F2H. A similar gel, revealed with Schiff's reagent, showed that proteins from fraction F2 and F2H were glycoproteins (Fig. 1D). The fraction F2H was denatured with guanidine chloride, reduced with DTT and alkylated with vinyl pyridine. After these treatments, two subunits (fractions S and L) were separated by reverse phase chromatography, initially on a C18 column (Fig. 2A), and were re-purified on a C2C18 column (Fig. 2B). The UV spectra showed a new peak at 254 nm after denaturing and alkylation treatments (inset Fig. 2A), indicating the production of a pyridylethyl cysteine (PEC) residue [1] in both fractions.

The isolated polypeptide chains were freeze-dried and submitted to tricine-SDS-PAGE and N-terminal sequencing. The molecular weights of the fractions were 5 kDa (fraction S) and 7.2 kDa (fraction L) as shown in Fig. 2C. The N-terminal partial sequence data of the small and large chains of 2S albumin are summarized in Table 1. These N-terminal sequences presented similarities with 2S albumin from *Ricinus communis*, *Curcubita maxima*, *Sesamum indicum*, *Solanum lycopersicum*, and *Helianthus annuus*. Sequence analysis revealed an important common feature: the conservation of four cysteine residues that are important for 2S albumin folding.

4.2. Allergic reactivity

Passive cutaneous anaphylaxis (PCA) is a profound localized allergic reaction triggered by allergen-induced cross-linking of Fc RI by the binding of an allergen-specific IgE placed beneath the skin. We used a PCA model to examine whether F2H could induce mast cell degranulation *in vivo*. The extravasation of Evans blue dye reflects increased local vascular permeability, a process that is dependent on mast cell release of histamine and serotonin upon degranulation. PCA tests challenged by F2H in rats sensitized with *J. curcas* 2S albumin were positive, indicating that this 2S albumin

is an allergenic protein (Fig. 3A). 2S albumin was thus identified as the first known allergen from *J. curcas*, and was denoted as Jat c 1 according to allergen nomenclature. Jat c 1 cross-reacted with allergenic 2S albumin from castor bean (Fig. 3B).

4.3. Mast cell degranulation

Several proteins from the 2S albumin family are known allergens [12,16,17,21,27]. Thorpe et al. [26] showed that Ric c 1 and Ric c 3 are the major castor bean allergens. To determine whether Jat c 1 from *J. curcas* cross-reacts with 2S albumin from castor bean, we used a mast cell degranulation assay *in vitro*. Table 2 shows the percentage of degranulation induced by Jat c 1 (52%) in rat mast cells when these cells were previously incubated with mice serum anti castor bean 2S albumin as the IgE source. Castor bean 2S albumin pool was utilized as a positive control and induced 65% of mast cell degranulation. In the absence of serum (IgE), the degranulation percentage induced by Jat c 1 was ~30%, a value similar to the negative control reported by Felix et al. [7] and Katayama et al. [10].

The level of histamine released by mast cells in contact with 2S albumin from *J. curcas* (Table 2) was notably higher than in the control without serum.

5. Discussion

We identified and sequenced a *J. curcas* allergen that belongs to the 2S albumin family. Its allergenic properties were demonstrated by PCA test, a type I allergic reaction *in vivo*. The protein has a molecular weight of around 12 kDa, is glycosylated, and promotes mast cell degranulation *in vitro*. The carbohydrate moiety seems important in immunoreactivity of serine protease, a major allergen of *Curvularia lunata*, [8] whoever it not important for castor bean allergens. So it is an important point to be investigated in Jat c 1. Given that most plant food allergens are pathogenesis-related

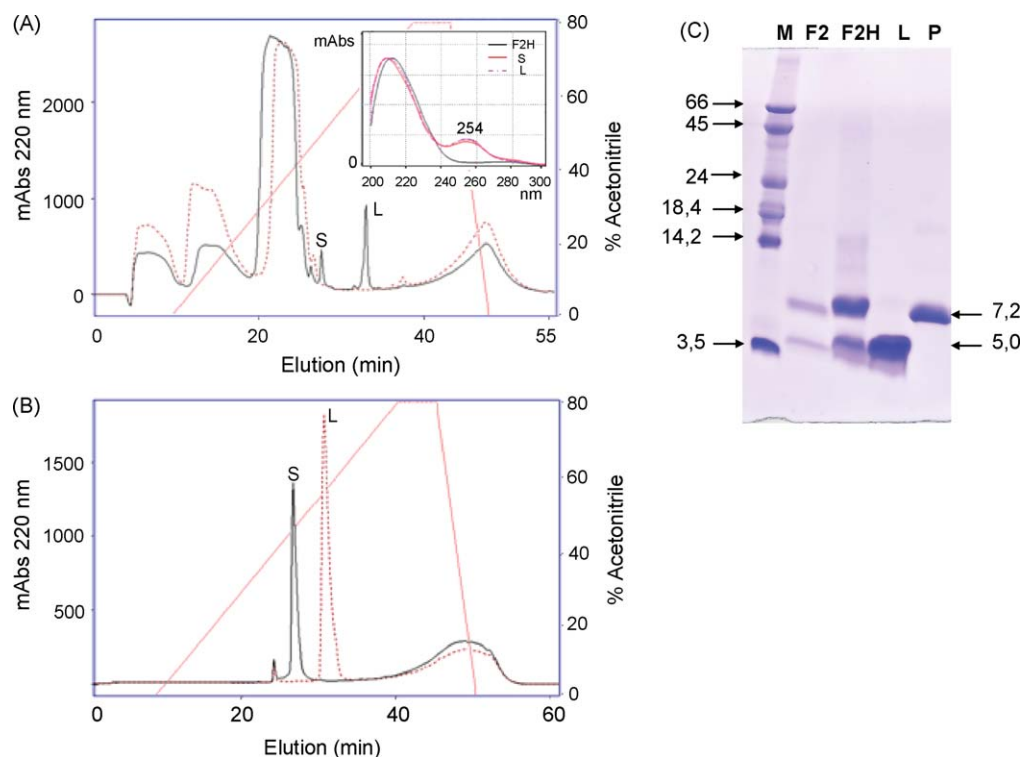


Fig. 2. (A) Chromatographic profiles from C18 fraction (F2H) treated with guanidine chloride, DTT and vinylpyridine on a C18 column. The dash line represents the blank reaction (without the 2S albumin). Peak S (small chain) and peak L (large chain). The oblique line represents the acetonitrile gradient. Inset in Fig. 1A represents spectra of F2H (native 2S albumin), peak S and L; (B) superposition of elution profiles of peaks S and L re-purified by a C2/C18 column. Elution profile was monitored at 220 nm; (C) tricine-SDS-PAGE of *J. curcas* fractions: F2, G-50 fraction; F2H, re-purified F2 by a C18 column; S and L, small and large chains obtained from F2H after pyridylethylation and separation on a C2C18 column. Protein load in each lane was 20 µg, except lane F2 that presents 3 µg. The gel was stained with Coomassie brilliant blue.

proteins, seed storage albumins and globulins, or α -amylase and protease inhibitors [15], and that several of them such as allergens from walnut (Jug r 1) [21], Brazil nut [6], castor bean (Ric c 1 and Ric c 3), soybean [12], and sesame [27] correspond to 2S albumin, it is not surprising that Jat c 1, a 2S albumin from *J. curcas*, is an important allergen in this euphorbia.

Our results confirm that Jat c 1 is an allergen that is present in *J. curcas* seeds and cross-reacts with the 2S albumin pool from castor bean, which is composed of the isoallergens Ric c 1 and Ric c 3 (11.2 and 12.0 kDa, respectively). This date indicated that an individual sensitized by allergens from castor bean (Ric c 1 and Ric c 3) could

become sensitive to 2S albumin from *J. curcas* (Jat c 1) and that the inverse condition may also be possible, suggesting that Jat c 1 has potential intrinsic allergenicity. Whether the IgE binding epitope is continuous or discontinuous requires further investigation. Since allergy to oleaginous seeds has emerged as an important clinical condition following an increase in the use of biodiesel, and given the risk due to cross-reactive allergens (as observed for allergens from *J. curcas* and *Ricinus communis*), advances in the identification and characterization of common aeroallergens and allergens from oleaginous seeds are necessary for the establishment of a specific therapy. Identification of allergenic components and subsequent

Table 1

Partial sequence data of *Jatropha curcas* 2S albumin, first small chain and second large chain. Data sequencing was performed by Edman degradation.

Jat c 1 (Small Chain)	1	VRDKCGEEAERTLXGCENYISQRR	25
EEF39549	49	CRQEIQRKDLSSCEQYIRQRR	72
EEF39550	46	CHQEFQQRKDLSSCEQYIRQRR	69
1PSY-A	18	CRQEVQRKDLSSCERYLRQRR	41
P01089	49	CRQEVQRKDLSSCERYLRQRR	72
SP Q39649.1	43	CRQMSAREELRSCEQYLQQR	63
AAK15088	44	CRQQLQGRQFRSCQRYLSQGR	64
AAB41308	48	CREQIQRQQLNHCQYLRQQR	69
Jat c 1 (Large Chain)	1	PREQVPRQCCNQALE	15
AAB25171	8	RQCCNQ	13
BAB39169	4	QCCNQ-LE	10
ABB60053	86	RQCCNQ	91
EEF39550	93	QCCNQ	97
EEF39549	96	QCCNQ	100
CAC81359	214	QCCNQ	218
1PSY-A	65	QCCNQ	69

X – Any amino acid. Bold values indicate cysteine conserved in small chain and conserved sequence in large chain; EEF39549 – 2S albumin precursor, putative [*Ricinus communis*]; EEF39550 – 2S albumin precursor, putative [*Ricinus communis*]; 1PSY-A – Chain A, Structure Of Ricc3, Nmr, 20 Structures; P01089 – RecName: Full = 2S albumin; AltName: Allergen = Ric c 1/3; Swiss-Prot – Q39649.1 – 2S albumin [*Cucurbita maxima*]; AAK15088 – 2S albumin [*Sesamum indicum*]; AAB25171 – Mabinlin II B chain = 2S albumin AT2S3 homolog; BAB39169 – 2S seed albumin-1 large subunit [*Solanum lycopersicum*]; ABB60053 – 2S albumin precursor isoform 3 [*Sesamum indicum*]; CAC81359 – Basic 2S albumin [*Helianthus annuus*].

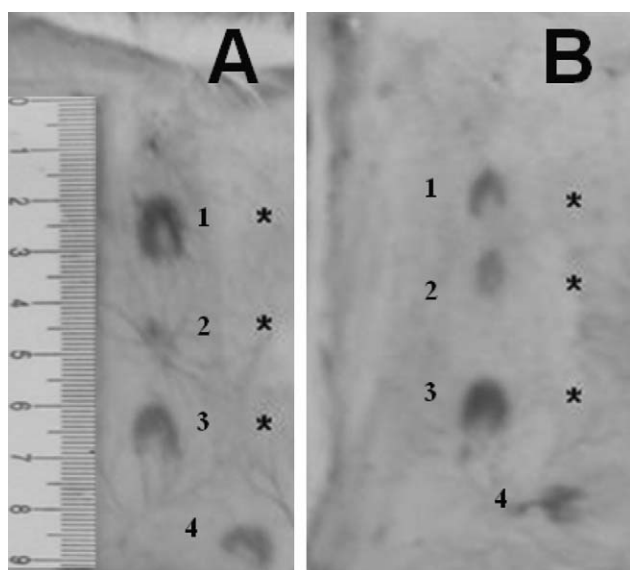


Fig. 3. Passive cutaneous anaphylaxis assay (PCA) Scale bar in mm. PCA reactions using Lou-M rats as receptors of anti-2S albumin sera from mice Balb/c. The sera were specific to *J. curcas* 2S albumin. (A) and (B) represent PCA reactions induced by F2H from *J. curcas* and 2S albumin from castor bean (*Ricinus communis*), respectively. 1–4 represent sera from four mice. (*) Sera without reaction in Lou-M rats.

Table 2

Percentage of mast cell degranulation and histamine released induced by *Ricinus communis* allergen isoforms and *Jatropha curcas* 2S albumin.

	Mast cell degranulation (%)	Histamine released (%)
Mast cell control (negative control)	28.9	4
2S albumin pool from <i>R. communis</i> + antiserum	63.4	60
2S albumin from <i>J. curcas</i> without antiserum	36.2	5
2S albumin from <i>J. curcas</i> + antiserum	51.7	56

sequence determination are important for mapping of the IgE binding site, and determination of T cell and B cell epitopes. Together, these data will facilitate improved diagnostics and investigation of specific therapeutics.

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References

- [1] Allen G. Sequencing of proteins and peptides. In: Bundon RH, Knipper PH, editors. 2nd ed., Laboratory techniques in biochemistry and molecular biology, vol. 9th, 2nd ed. The Netherlands, Amsterdam: Elsevier; 1989.
- [2] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389–402.
- [3] Bashir MEH, Hubatsch I, Leinenbach HP, Zeppezauer M, Panzani RC, Hussein IH. Ric c 1 and Ric c 3, the allergenic 2S albumin storage proteins of *Ricinus communis*: complete primary structures and phylogenetic relationships. *Int Arch Allergy Immunol* 1998;115:73–82.
- [4] Bradford MM. A rapid sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1976;72:248–54.
- [5] Braga F, Mota I. Homologous passive cutaneous anaphylaxis (PCA) in mice and heterologous PCA induced in rats with mouse IgE. *Immunology* 1976;30:655–69.
- [6] Breiteneder H, Mills ENC. Plant food allergens – structural and functional aspects of allergenicity. *Biotechnol Adv* 2005;23:395–9.
- [7] Felix SP, Mayerhoffer RO, Damatta RA, Vericimo MA, Nascimento VV, Machado OLT. Mapping IgE-binding epitopes of Ric c 1 and Ric c 3, allergens from *Ricinus communis*, by mast cell degranulation assay. *Peptides* 2008;29:497–504.
- [8] Gupta R, Sharma V, Sridhara S, Singh BP, Arora N. Identification of serine protease as a major allergen of *Curvularia lunata*. *Allergy* 2004;59:421–7.
- [9] Heller J. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. Rome: Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute; 1996.
- [10] Katayama G, Nabe T, Fujii M, Kohno S. Mast cell hyperplasia induced by multiple challenge with cedar pollen in sensitized guinea pigs. *Inflamm Res* 2005;54:370–4.
- [11] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–5.
- [12] Lin J, Shewry PR, Archer DB, Beyer K, Niggemann B, Haas H, et al. The potential allergenicity of two 2S albumins from soybean (*Glycine max*): a protein microarray approach. *Inter Arch Allerg Immunol* 2006;141:91–102.
- [13] Machado OLT, Marcondes JA, de Souza-Silva F, Hansen E, Ribeiro PD, Vericimo M, et al. Characterization of allergenic 2S albumin isoforms from *Ricinus communis* seeds. *Allergologie* 2003;26:45–51.
- [14] Machado OLT, Silva JG. An allergenic 2S storage protein from *Ricinus communis* seeds which is part of the 2S albumin precursor predicted by c-DNA data. *Braz J Med Biol Res* 1992;25:567–82.
- [15] Makkar HPS, Becker K. Plant toxins and detoxification methods to improve feed quality of tropical seeds. *Rev Asian Australas J Anim Sci* 1999;12:467–80.
- [16] Mills ENC, Madsen C, Shewry PR, Wichers HJ. Food allergens of plant origin – their molecular and evolutionary relationships. *Trends Food Sci Technol* 2003;14:145–56.
- [17] Moreno FJ, Rubio LA, Olano A, Clemente A. Uptake of 2S albumin allergens, Ber e 1 and Ses i 1, across human intestinal epithelial Caco-2 cell monolayers. *J Agric Food Chem* 2006;54:8631–9.
- [18] Ordman D. An outbreak of bronchial asthma in South Africa, affecting more than 200 persons, caused by castor bean dust from an oil-processing factory. *Int Arch Allergy Appl Immunol* 1955;7:10–24.
- [19] Prueksakorn K, Gheewala SH. Full chain energy analysis of biodiesel from *Jatropha curcas* L. in Thailand. *Environ Sci Technol* 2008;42:3388–93.
- [20] Rakshita KD, Darukeshwaraa J, Rathina Rajb K, Narasimhamurthy K, Saibabab P, Bhagya S. Toxicity studies of detoxified *Jatropha* meal (*Jatropha curcas*) in rats. *Food Chem Toxicol* 2008;46:3621–5.
- [21] Robotham JM, Teuber SS, Sathe SK, Roux KH. Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen Jug r 1. *J Allergy Clin Immunol* 2002;109:143–9.
- [22] Schägger H, von Jagow G. Tricine–sodium dodecyl sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;166:368–79.
- [23] Silva Jr JG, Machado OLT, Izumi C, Padovan JC, Chait BT, Mirza UA, et al. Amino acid sequence of a new 2S Albumin from *Ricinus communis* which is part of a 29-kDa precursor protein. *Arch Biochem Biophys* 1996;36:10–8.
- [24] Stirpe F, Pession-Brizzi A, Lorenzoni E, Strocchi P, Montanaro L, Sperti S. Studies on the proteins from the seeds of *Croton tiglium* and of *Jatropha curcas*. Toxic properties and inhibition of protein synthesis in vitro. *Biochem J* 1976;156:1–6.
- [25] Tapanes NCO, Aranda DAG, Carneiro JWM, Antunes OAC. Transesterification of *Jatropha curcas* oil glycerides: theoretical and experimental studies of biodiesel reaction. *Fuel* 2008;87:2286–95.
- [26] Thorpe SC, Kemeny DM, Panzani RC, McGurl B, Lord JM. Identification of the major allergens in castor bean seeds. *J Allergy Clin Immunol* 1988;82:67–72.
- [27] Wolff N, Cogan U, Admon A, Dalal I, Katz Y, Hodos N, et al. Allergy to sesame in humans is associated primarily with IgE antibody to a 14 kDa 2S albumin precursor. *Food Chem Toxicol* 2003;41:1165–74.
- [28] Youn-Young S, Janitha P, Wanasundara D. Quantitative detection of allergenic protein Sin a 1 from yellow mustard (*Sinapis alba* L.) seeds using enzyme-linked immunosorbent assay. *J Agric Food Chem* 2008;56:1184–92.