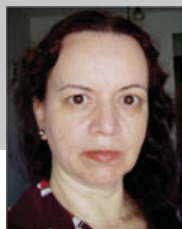


Letter to the Editor



Glutamic acid-rich proteins in cassava (*Manihot esculenta* Crantz) storage roots

We have read the article entitled “Novel allergens from ancient foods: Man e5 from manioc (*Manihot esculenta* Crantz) cross reacts with Hev b5 from latex” recently reported by Santos et al. [1]. Although it is a very interesting article, we detected some points that must be considered, since we have also worked on isolation and characterization of cassava proteins and genes, with previous data related to results presented by the authors.

(i) In a previous study, Santos et al. [2] have reported cassava proteins reacting with IgE from patients allergic to cassava, among them, a 30 kDa protein identified as Pt2L4, a glutamic acid-rich protein (GenBank AAM55492.1). In a more recent paper, Santos et al. [1] have reported the cloning and expression in bacterial system of the cassava Man e5 allergen (GenBank AEE98392.1), which is different from cassava glutamic acid-rich protein (GenBank AAM55492.1) in only three amino acid residues. To date, GenBank AAM55492.1 accession corresponds to sequence of the allergenic-related protein Pt2L4, an alcohol-soluble protein isolated from tissue layer 4 of cassava storage roots by our group. It is a highly acidic protein with a predicted molecular weight of 18.8 kDa, however in SDS-PAGE the Pt2L4 was visualized as a 32 kDa band. Pt2L4 protein is an homologue to the Hev b 5 allergen from rubber tree (*Hevea brasiliensis*) and its transcripts are differentially accumulated in tissue layers of cassava storage root [3–5].

(ii) In the *Material and Methods*, Santos et al. [1] have informed that the primers used to isolate the full-length sequence of Man e5 were designed based on the manioc glutamic acid-rich protein Pt2L4 (GenBank AAP57707.1). However, the GenBank AAP57707 accession refers to the amino acid sequence of C54 glutamic acid-rich protein

[6], which showed 60–62% of identity with Pt2L4 protein (GenBank AAM55492.1) [4, 6]. According to the primers sequences available in the Supporting Information, these primers were probably based on GenBank AY101376.1 accession, which corresponds to the full-length cDNA sequence of Pt2L4 protein (GenBank AAM55492.1). The sequences of forward (5'ATGGCTACTGCTGAGG3') and reverse (5'TCACTCAGTCTTCTC3') primers without *Nde*I and *Xho*I sites match with 5' and 3' ends of Pt2L4 cds, with exception that the stop codon TAA was replaced by TGA, the last nucleotides of the Man e5 cds (GenBank JF710639). Since Man e5 is not totally identical to the other well-characterized glutamic acid-rich proteins in cassava plant, would be interesting to isolate the full-length cDNA by RACE assays or cDNA library in order to confirm this new sequence. This suggestion can be supported by a BLAST search of Pt2L4 protein (AAM55492.1) against the Phytozome Cassava Database (<http://www.phytozome.net/cassava>), where this sequence matches with the cassava4.1_017439m, cassava4.1_018577m and cassava4.1_018170m accessions.

(iii) In the Results, Santos et al. [1] have reported that the amino acid sequence of Man e5 deviated from glutamic acid-rich protein GenBank AAM55492.1 sequence in three positions, i.e. P18S, A116T and V122E. However, according to the alignment between these two proteins sequences (Fig. 1, below) there are five different amino acids: E57G, A58G, A116T, V122E and S148P. While, in comparison to GenBank ABY19572 there are three deviated amino acids residues, i.e. E17K, S148P, and an additional G at position 91 within this accession.

(iv) In addition, it was detected that the Man e5 sequence used in analyses performed by Santos et al. [1] does not exactly correspond to the GenBank AEE98392.1 sequence. The amino acids 116T and 122E were correctly shown in the Man e5 sequence used in alignment with Hev b5 (Fig. 1A of cited article), but 57G, 58G and 148P were replaced by 57E, 58A and 148S, respectively. Also, at position 18 a proline was replaced by a serine. These changes were also observed in Man

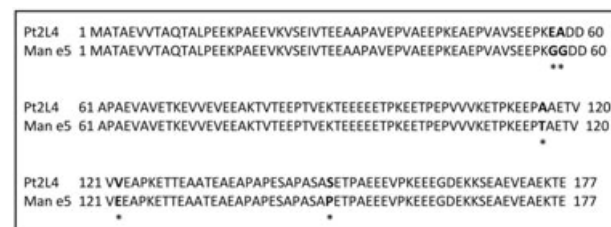


Figure 1. Alignment between the Pt2L4 (GenBank AAM55492.1) and Man e5 (GenBank AEE98392.1) glutamic acid-rich proteins of cassava. Different amino acids residues are in bold and indicated by asterisks under the alignment.

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e5 sequence used in secondary structure prediction (Fig. 3B of cited article).

In conclusion, this letter gently calls the readers' attention for some examples of incorrect informations within the article reported by Santos et al. [1], including citations of data about cassava glutamic acid-rich proteins sequences available in GenBank Databases.

The authors have declared no conflict of interest.

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