

Molecular cloning and characterization of Fag t 2: a 16-kDa major allergen from Tartary buckwheat seeds

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An increased consumption of Tartary buckwheat (*Fagopyrum tataricum*, TB) has been observed because of its richer health beneficial nutrition components than common buckwheat (*F. esculentum*, CB). However, because the allergen proteins occur in TB seeds, incidences of buckwheat allergy have been frequently reported. Ingestion of buckwheat foods can cause immediate hypersensitivity reactions (IHR) (1, 2). The 16-kDa allergen is a major allergen in CB seeds because of its anti-pepsin activity associated with IHR (3). Here, we reported the identification and characterization of one 16-kDa major allergen in TB seeds, namely

This report presents the cloning and characterization of a major allergen, Fag t 2, from the Tartary buckwheat (*Fagopyrum Tataricum*) and demonstrated its IgE-binding capacity, pepsin digestion resistance, thermal stability and its preferential distribution in the embryo of the seeds.

Fag t 2, assigned by the I.U.I.S. Allergen Nomenclature Sub-committee.

A cDNA library was constructed from developing TB seeds, and approximately 1000 cDNA clones were sequenced. One cDNA clone was identified with a high abundance (1.1%) and a high degree of DNA sequence homology (82%) to the gene for CB 16-kDa allergen. Full-length cDNA of this putative Fag t 2 gene from TB was obtained (GneBank accession No. HQ829975). Functional predictions indicated that this gene encoded a protein of 149 amino acid residues with a putative signal peptide, which belonged to the 2S albumin family. The deduced protein was predicted to have four α -helices with six possible epitopes located in α -helices. The 384 bp putative mature Fag t 2 coding sequence was subcloned into the pET47b expression vector, and the recombinant protein (rFag t 2) was overexpressed in *E. coli* BL21star (DE3) strain and purified via immobilized metal affinity chromatography. A native Fag t 2 (nFag t 2) was purified from defatted TB seed powder. The

N-terminal amino acid sequencing (RDEGFDDLGETQM) of nFag t 2 and MALDI-TOF analysis confirmed the identity of amino acid sequences between nFag t 2 and rFag t 2.

In vitro IgE immunoblotting showed that both nFag t 2 (Fig. 1A) and rFag t 2 (Fig. 1B) were able to react with two positive sera from the patients allergic to buckwheat, but not with the control serum. Competitive ELISA (4) (Table 1) revealed a similar inhibitory efficiency of rFag t 2 and nFag t 2, suggesting that they both had the same specific IgE-binding activity. In ELISA tests, after being heated at 100°C for 150 min, rFag t 2 and crude nFag t 2 had 93.4% and 95.1% IgE-binding capacity, respectively, when compared with that in no-heated Fag t 2 control samples (data not shown). In addition, pepsin digestion tests demonstrated that both the TB nFag t 2 and rFag t 2 were resistant to digestion (data not shown).

Western blotting analysis of total seed protein, albumin, globulin,

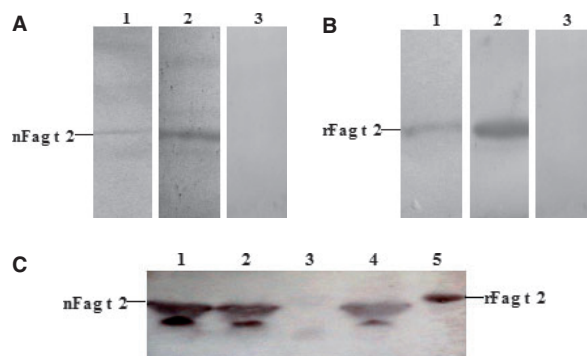


Figure 1 IgE immunoblotting (A,B) and Western blot (C) analyses of Fag t 2. nFag t 2 = native Fag t 2; rFag t 2 = recombinant Fag t 2. In A and B, lanes 1 and 2 = IgE immunoblotting with two positive sera (1 and 2) from patients allergic to buckwheat, respectively; lanes 3 = IgE immunoblotting with control sera. In C, lanes 1–5 = seed total protein, albumin, globulin, seed total protein extracted by HCl and rFag t 2, respectively.

Table 1 IgE-binding capacity of Fag t 2 by competitive ELISA*

Samples	Serum no. 1		Serum no. 2		Control	
	A	I	A	I	A	I
TB seed total protein	0.428	n/a	0.469	n/a	0.0	n/a
rFag t 2	0.258	39.5	0.273	41.9	0.0	n/a
nFag t 2	0.249	41.6	0.267	43.0	0.0	n/a

*Inhibition ratio I = $[(A_{\text{total protein}} - A_{\text{rFag t 2}} \text{ or } A_{\text{nFag t 2}}) / A_{\text{total protein}}] \times 100$.

A = colorimetric intensity (OD₄₅₀). n/a = not applicable. Buckwheat-specific IgE values examined *in vitro* for the no. 1 and no. 2 serum samples were 17.2 and 46.9 IU/ml, respectively.

acid-soluble protein extracted by HCl (pH 2.0) and rFag t 2 with rabbit anti-rFag t 2 antibodies (Fig. 1C) suggested that nFag t 2 was expressed in TB seeds abundantly and Fag t 2 only presents in albumin and is acid stable. Meanwhile, Western blotting of embryo and endosperm proteins revealed that Fag t 2 was restricted to embryo, which was consistent with the previous observations (5). One extra band was detected below the 16-kDa target (Fig. 1C), which could correspond to the TB 10-kDa allergen.

In summary, we have obtained the full-length cDNA of the protein of TB 16-kDa major allergen protein Fag t 2. We showed that Fag t 2 belongs to the 2S albumin family preferentially expressed in the embryo of TB seeds. Fag t 2 was resistant to pepsin digestion and heating. Our results will contribute to buckwheat allergen-specific immunotherapy and diagnosis (6).

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Conflict of interest

The authors declare no conflicts of interest.

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