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Epitope Analysis of the Major Allergic Protein Fag e 1 from Autogamous Common Buckwheat

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Objectives

In the present study, we focused on the molecular characterization of Fag e 1 for the purpose of developing hypoallerenic buckwheat. Fag e 1 cDNA was isolated from autogamous common buckwheat and its antigenicity confirmed by immunoblotting using recombinant protein expressed in *E.coli.* The derived amino acid sequence from Fag e 1 cDNA has been used to construct the synthetic peptides, and we have identified the major IgE binding epitopes in this allergenic protein. We have also identified the amino acids within each of the IgE binding. Our results will be used in future attempts to genetically decrease the allergenic activity of buckwheat storage proteins by modifying its composition or IgE binding sites.

Materials and Methods

The buckwheat strain has 99% putative genetic background of *F. esculentum* and expresses stable self-compatibility.

Two oligonucleotides, FeAg22kDa-F (GAGAGAGCTCTGGATTGCGTTCTG) plus Sac 1 restriction enzyme cassette and FeAg-R (AAGTCTAGACGAGAATTCACTCTTTTATTGAC) plus Xba 1 restrction enzyme cassette derived from the 5' and 3' ends of Fag e 1 cDNA, respectively (Nair and Adachi, 1999). Allergen assay of recombinant Fag e 1 by immunoblotting. The purified fusion protein was cleaved beween the TRX-His-Tag and Fag e 1 by treatment with enterokinase and electroblotted onto PVDF membrane after separation on SDS-PAGE. The critical amino acids of Fag e 1 IgE epitopes were identified by synthesizing multiple peptides with single amino acid chages at each position.

Results and Discussion

A major allergenic protein of buckwheat is Fag e 1. We isolated the respective cDNA, coding for a 22 kDa protein, from a recently developed autogamous strain of common buckwheat and confirmed its immunoglobulin E (IgE)-binding activity using recombinant Fag e 1 and sera of allergic patients. The derived amino acid sequence from Fag e 1 cDNA was used to synthesize an overlapping peptide library on nitrocellulose membranes for the determination of the Fag e 1 epitopes. We identified eight epitopes and the critical amino acids for IgE-binding within the epitopes. This epitope analysis of a major allergenic protein of buckwheat should help therapeutic efforts and aid in the development of hypoallergenic buckwheat.

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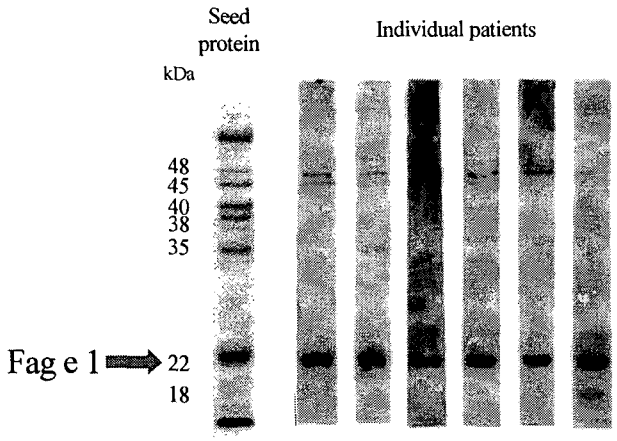

Fig.) Immunoblotting by using patient's serum for seed strage protein extracted from BC_6F_2 (self-fertilization lines).

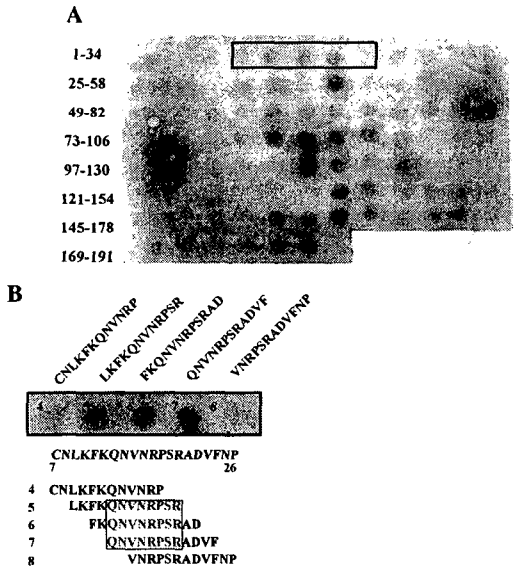

Fig.) Multiple IgE-binding regions identified on the Fag e 1 allergen and epitope determination.
