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Wild Buckwheat Is Unlikely to Pose a Risk to Buckwheat-Allergic Individuals

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Abstract

Buckwheat (*Fagopyrum esculentum*) is a commonly allergenic food especially in Asia where buckwheat is more commonly consumed. Wild buckwheat (*Polygonum convolvulus*, recently changed to *Fallopia convolvulus*) is an annual weed prevalent in grain-growing areas of the United States. Wild buckwheat is not closely related to edible buckwheat although the seeds do have some physical resemblance. A large shipment of wheat into Japan was halted by the discovery of the adventitious presence of wild buckwheat seeds over possible concerns for buckwheat-allergic consumers. However, IgE-binding was not observed to an extract of wild buckwheat using sera from 3 buckwheat-allergic individuals either by radio-allergosorbent test inhibition or by immunoblotting after protein separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Furthermore, the extract of wild buckwheat was not detected in a buckwheat enzyme-linked immunosorbent assay developed with antisera against common buckwheat. Thus, wild buckwheat is highly unlikely to pose any risk to buckwheat-allergic individuals. The common names of plants should not be a factor in the risk assessment for possible cross-allergenicity.
Keywords: allergy, buckwheat, grain, wild buckwheat

Introduction

Buckwheat (*Fagopyrum esculentum*) is a commonly allergenic food in Asian countries probably owing to the frequent consumption of buckwheat noodles (Ebisawa et al. 2003). Buckwheat appears on the list of priority allergenic foods in Japan and South Korea (Taylor and Hefle 2005) but not other countries where buckwheat allergy is less frequently encountered. Buckwheat allergy can also be quite severe for some affected patients (Noma et al. 2001; Moneret-Vautrin et al. 2005; Imamura et al. 2008). Enzyme-linked immunosorbent assays (ELISAs) for the detection of undeclared buckwheat residues in foods have been developed to support labeling regulations in Japan (Akiyama et al. 2004; Panda et al. 2010).

Wild buckwheat (*Polygonum convolvulus*; *Fallopia convolvulus*) is an annual weed prevalent in the midwestern and northern plains of the United States. It frequently infests fields of wheat, soybeans, and other grains (Zollinger et al., 2006). It is distantly related to common buckwheat (*F. esculentum*). Wild buckwheat and common buckwheat are in the same genetic family (*Polygonaceae*) but are distinct at the genus level. The use of the name, "buckwheat," for both of these distantly related plants has the potential to cause confusion for those with buckwheat allergy who must practice avoidance diets and for public health officials attempting to protect such consumers from undeclared buckwheat in foods. Although not closely related, these 2 types of seeds do have a somewhat similar appearance as both are triangular and dark in color, although wild buckwheat seeds are smaller.

Recently, the offloading of a large shipment of wheat into Japan was halted by the visual discovery by Japanese inspectors of the adventitious presence of wild buckwheat seeds mixed with the wheat grain. This study was conducted to determine if wild buckwheat would cross-react with buckwheat in the buckwheat ELISA and to determine if wild buckwheat would bind to serum IgE from buckwheat-allergic individuals.

Materials and Methods

Buckwheat, wild buckwheat, and preparation of extracts

Buckwheat flour and buckwheat seeds were obtained from a local retail outlet. Wild buckwheat seeds were obtained from the grain company whose shipment was embargoed in Japan. Buckwheat seeds and wild buckwheat seeds were crushed using dedicated, individual blender jars, and blades. Extracts of buckwheat flour, buckwheat seeds, and wild buckwheat seeds were prepared for the ELISA by mixing the flour or crushed grain at a ratio of 1:20 (w/v) with phosphate buffered saline (0.01 M sodium phosphate in 0.85% sodium chloride, pH 7.4) (PBS) + 1% nonfat dry milk (NFDM) with shaking at 60 °C for 2 h in a water bath and were clarified by centrifugation for 30 min at 2000× *g*. A second set of extracts of buckwheat flour, buckwheat seeds, and wild buckwheat seeds were prepared by rocking 1:10 (w/v) with PBS + 0.02% sodium azide, pH 7.4 overnight at 4 °C. The extracts were clarified by centrifugation for 30 min at 3000× *g*. The protein concentrations of the extract were determined by the method of Lowry et al. (1951).

Buckwheat ELISA

The buckwheat ELISA was performed as described by Panda et al. (2010) using rabbit anti-buckwheat antisera as the capture antibody and goat anti-buckwheat antisera as the detector antibody.

Human sera

Sera were obtained from 3 buckwheat-allergic individuals from a collection of sera from food-allergic subjects maintained

by the Food Allergy Research and Resource Program at the University of Nebraska–Lincoln obtained through an approved Institutional Review Board protocol. These sera from North American subjects had specific IgE scores for buckwheat ranging from 12.4 to 43.8 kUA/L (kilo units buckwheat specific IgE per liter serum), a score that is considered as indicative of very strong IgE binding. Additionally, the 3 allergic individuals had convincing clinical histories including projectile vomiting, abdominal pain, diarrhea, asthma, swelling of mouth, throat and face, urticaria, and anaphylaxis associated with the ingestion of buckwheat. One of these subjects had no other food allergies, while the other two subjects were allergic to both walnut and buckwheat by history and specific IgE results. All of these subjects had various inhalant allergies by history including pollen, animal dander, and dust mite allergies.

RAST inhibition assay

A radio-allergosorbent test (RAST) inhibition assay was used to evaluate the ability of the extract of wild buckwheat seeds to compete with buckwheat proteins bound to solid phase for binding of IgE from the pooled sera of buckwheat-allergic individuals using a protocol essentially as described in Hefle et al. (1994). Buckwheat proteins from the 1:10 extract of buckwheat flour were bound to a solid phase (cyanogen bromide-activated Sepharose® 4B, Amersham Biosciences Corp., Piscataway, N.J., U.S.A.) which was suspended in RAST-buffer (0.05 M sodium phosphate, 2.5% sodium chloride, 0.2% bovine serum albumin, 0.5% Tween 20, 0.05% sodium azide, pH 7.5) at the rate of 3% (v/v) of swollen solid phase in RAST buffer. Serial dilutions of a 1:20 buckwheat flour extract, a 1:20 buckwheat seed extract, and a 1:20 wild buckwheat seed extract were separately mixed with 0.5 mL of a 3% concentration of the suspended buckwheat solid phase and 0.1 mL of a 1:5 dilution of the pooled buckwheat-allergic sera. After incubation and removal of unbound human sera by washing, the tubes of solid phase were incubated overnight with antihuman IgE labeled with Iodine 125 (I-125). Unbound anti-IgE was removed by washing. The amount of IgE bound to the solid phase was determined by measuring the residual radioactivity of the solid phase with a sodium iodide scintillation detector. The percent inhibition of IgE binding was calculated with the use of values from buckwheat solid phase samples without inhibitor protein as a measurement of maximal binding.

Electrophoresis and blotting

The extracts prepared with PBS + 0.02% sodium azide were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 10% to 20% gradient gels. Wells were loaded with 10 μg protein except in the case of the wild buckwheat seed extract that was loaded at 5 μg (maximum amount based on volume of well). A limited quantity of wild buckwheat seeds was available so concentration of the resultant extract was not possible. Electrophoresis was performed according to the manufacturer's instructions for the Mini-Protean® II dual slab cell (Bio-Rad Laboratories, Hercules, Calif., U.S.A.). Separated proteins were transferred to polyvinyl difluoride (PVDF) by electroblotting according to the manufacturer's directions for the Mini Trans-Blot® electrophoretic transfer cell (Bio-Rad Laboratories**)**. PVDF blots were blocked with RAST buffer and then incubated overnight either with control serum or serum samples from the buckwheat-allergic subjects diluted 1:10 in RAST buffer. Blots were then washed with RAST buffer and then incubated overnight with antihuman IgE labeled with I-125. Blots were washed to remove unbound antihuman IgE, allowed to dry, mounted, and placed between transparencies and exposed to X-ray film for 48 to 96 h at −80 °C. Protein bands binding human IgE were visualized after developing the film. One blot was not blocked but stained with India ink to confirm protein transfer to the blots. India ink staining was achieved by washing the blot 2 × 5 min in PBS plus 0.1% Tween 20 and then staining for 15 min to 24 h with 0.1% India Ink (Pelikan, Hannover, Germany) in PBS with 0.1% Tween 20.

Results and Discussion

The extract of wild buckwheat (1:20 w/v) was not detected in the buckwheat ELISA at the lower limit of quantitation of the ELISA of 2 ppm. Thus, the IgG antisera used in the buckwheat ELISA was not cross-reactive with any proteins present in the extract of the wild buckwheat seeds. Because the RAST inhibition assay evaluates competitive binding to IgE antibodies from the sera of buckwheat-allergic individuals, the results of this assay are much more useful in the assessment of the potential allergenicity of wild buckwheat seeds. As shown in Figure 1, the extracts of common buckwheat flour and common buckwheat seeds were able to compete strongly for binding to solid-phase buckwheat proteins. By comparison, the extract of wild buckwheat seeds exhibited a very low level of inhibition indicating negligible binding of buckwheat specific IgE to proteins in the wild buckwheat extract.

On immunoblots (Figure 2), none of the 3 sera from buckwheat-allergic individuals recognized proteins from wild buckwheat that were separated by SDS-PAGE and transferred to the immunoblots. In contrast, all 3 sera recognized proteins in the buckwheat flour lanes, although not necessarily the same proteins in each case. While research on the identification of buckwheat allergens has been somewhat limited, multiple allergens are known to exist (Nair and Adachi, 1999) and thus the diversity of IgE binding to buckwheat proteins observed with these 3 sera is not surprising. No binding was observed with serum from a nonallergic negative control (data not shown). As noted in Fig. 2, the degree of protein staining in Lane 4 (wild buckwheat

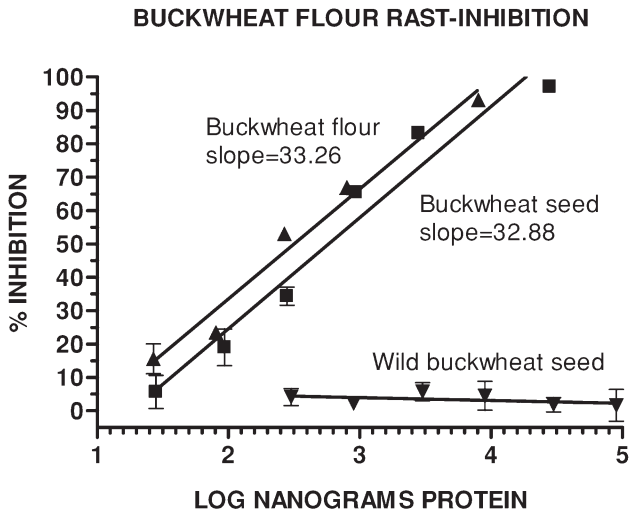

Figure 1. Comparison of wild buckwheat with common buckwheat and buckwheat flour to inhibit IgE-binding from human sera of buckwheat-allergic individuals as shown by RAST inhibition.

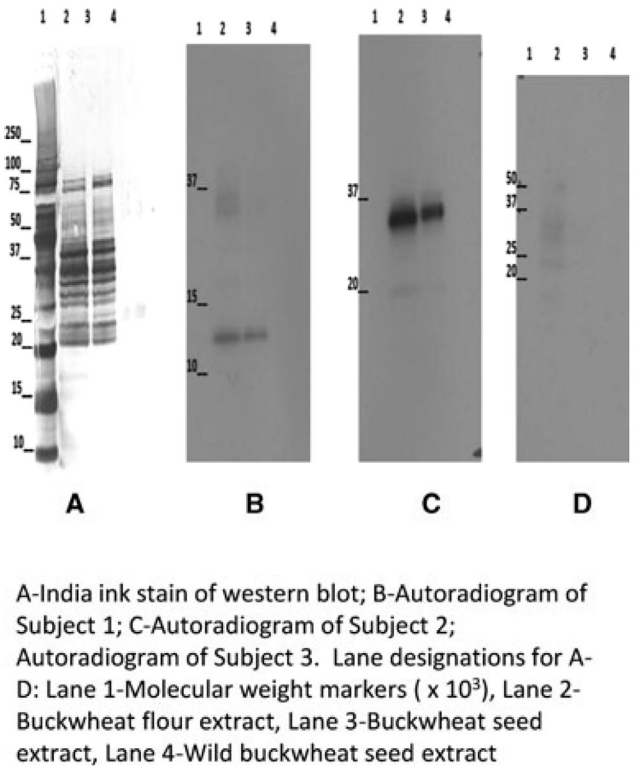

Figure 2. SDS-polyacrylamide gel electrophoresis of molecular weight markers (Lane 1), buckwheat flour (Lane 2), buckwheat seed extract (Lane 3), and wild buckwheat seed extract (Lane 4). Protein stain is shown on the leftmost panel while immunoblotting with sera from 3 different buckwheat-allergic subjects is shown on the following 3 panels.

extract) obtained with India ink is less than anticipated by the comparative amounts of protein loading of the wells in Lanes 2 and 3 (10 compared with 5 μg). This may reflect the comparative ability of India ink to bind to the proteins from the different extracts or to the possible existence of a greater diversity of protein in the wild buckwheat extract.

Based upon these results, wild buckwheat (*P. convolvulus*) is highly unlikely to present a risk to buckwheat-allergic individuals. The profound difference in IgE binding between common buckwheat (*F. esculentum*) and wild buckwheat is sufficient to conclude that additional evidence of the lack of allergenicity of wild buckwheat is not needed. No evidence of the allergenicity of wild buckwheat exists in the published clinical literature despite the high likelihood that some intake of wild buckwheat seeds is likely to have occurred due to grain contamination. No reason exists to suggest that wild buckwheat seeds might pose a risk to buckwheat-allergic individuals. Wild buckwheat is not closely related to edible buckwheat even though the seeds of wild buckwheat bear some physical resemblance of edible buckwheat seeds. The common names of plants should not be a factor in the risk assessment for possible cross-allergenicity. Instead, the botanical relationships are more likely to predict potential risk.

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