

Invited Review

Understanding buckwheat allergies for the management of allergic reactions in humans and animals

Rie Satoh^{*1)}, Erika Jensen-Jarolim^{2,3)} and Reiko Teshima⁴⁾

¹⁾ *Functionality Evaluation Unit, Division of Food Function Research, Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan*

²⁾ *Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria*

³⁾ *The Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, University of Vienna, Veterinärplatz 1, 1210, Vienna, Austria*

⁴⁾ *Division of Food Safety, Faculty of Veterinary Medicine, Okayama University of Sciences, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan*

Buckwheat allergy is an immediate hypersensitivity reaction that includes anaphylaxis mediated by specific IgE antibodies. Several IgE-binding proteins in common buckwheat have been reported to be possible clinically relevant buckwheat allergens. Although common buckwheat is popularly consumed in Asia, buckwheat allergy is becoming a serious problem not only in Asia but also in Europe. In addition, common buckwheat has also been found to be a causative agent of allergic symptoms in animals. In recent years, in addition to conventional food allergy testing methods, the development of component-resolved diagnosis (CRD) has improved the diagnostic accuracy of food allergy. The identification of allergens is essential for the construction of CRD. In this review, we introduce the different types of buckwheat allergens and discuss how each buckwheat allergen contributes to the diagnosis of buckwheat allergy. We also present the analysis of buckwheat allergen that will help reduce the allergenicity of common buckwheat and reduce buckwheat allergen molecules. These findings may be beneficial in overcoming buckwheat allergies in humans and animals.

Key Words: allergen component, allergen molecules, anaphylaxis, buckwheat allergen, buckwheat allergy, component-resolved diagnosis.

Introduction

Food allergies are a serious health concern, and the frequencies of these allergies are increasing globally. Food allergy is defined as 'an adverse reaction to food in which immunologic mechanisms have been demonstrated' (Muraro *et al.* 2014). Ingestion, inhalation or skin contact with causative food components can trigger allergic responses in individuals. The causative components of allergic reactions are mostly immunoglobulin E (IgE)-binding proteins called allergens or allergen components. When an organism is first exposed to allergy-causative foods, food allergen-specific IgE antibodies are produced; these antibodies bind to high-affinity IgE receptors, also known as Fc epsilon receptor 1 (FcεRI), on the surface of mast cells in the blood. Mast cells are effector cells in IgE-

mediated immediate hypersensitivity and allergic conditions (Kawakami and Galli 2002). Second exposure to the causative foods causes the allergens to bind to specific IgE antibodies, leading to cross-linking of FcεRI, and the mast cells become activated. The activated mast cells evoke the degranulation of granules that store inflammatory chemical mediators, such as histamines and proteases, and the activated mast cells produce and release cytokines and chemokines. Consequently, the released inflammatory mediators induce the clinical symptoms of allergic reactions.

Common buckwheat (*Fagopyrum esculentum* Moench) is a highly nutritious pseudocereal, comprising nutrients such as proteins, dietary fibre, vitamins and minerals (Gimenez-Bastida and Zielinski 2015). Common buckwheat also contains rutin, which is a functional ingredient with antioxidant capacity. Common buckwheat is eaten around the world as a variety of dishes such as noodles, dumplings, galettes (buckwheat pancakes), soups, porridges, cookies and sausages. Common buckwheat husks are used for pillow fillings. Buckwheat allergy is characterised by severe and critical symptoms induced by ingestion

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*Corresponding author (e-mail: riesatoh@affrc.go.jp)

or inhalation of even a small amount of the common buckwheat flour or common buckwheat-containing food products (Horesh 1972, Nakamura *et al.* 1974, Wieslander 1996). Severe allergy to common buckwheat was first reported in 1909 (Smith 1909). Common buckwheat is also added to animal feed for the purpose of nutrient addition (Keles *et al.* 2018), and its husk is used as bedding material to add comfort to the animal breeding environment. However, recently, buckwheat allergy has also been reported in horses (Einhorn *et al.* 2018).

The principle of food allergy treatment is the avoidance of the causative foods after a correct diagnosis. Excessive avoidance of foods that are suspected to be causative food allergens may lead to poor growth and nutrient deficiency. Therefore, an accurate diagnosis of food allergy is necessary to avoid the development of allergic symptoms by eliminating the minimum causative foods from the diet. Conventional allergy diagnosis is mainly performed using *in vitro* allergen-specific IgE (sIgE) tests with patients' sera, skin prick tests (SPTs) and oral food challenge (OFC) tests in addition to medical interviews (Muraro *et al.* 2014). The first choice for allergy diagnosis is sIgE tests and/or SPT due to their practicability, although these tests show lower positive predictive accuracy. The OFC test is the most reliable method for the diagnosis of food allergy, and it is mainly performed for definitive diagnosis and confirmation of tolerance acquisition. However, the OFC test is associated with a substantial risk of causing serious allergic reactions. The OFC test can be used for the accurate diagnosis of food allergy using evidence on the amount of safe and ingestible foods. A probability curve may help predict the reactivity to a food in patients with food allergies (Sampson 2001). An IgE crosslinking-induced luciferase expression (EXiLE) test is a convenient and sensitive method for detecting IgE crosslinking-induced mast cell activation (Nakamura *et al.* 2010). Component-resolved diagnosis (CRD) is an *in vitro* diagnostic method, which provides an allergic patient's individual IgE-reactivity profile for each allergen component (Matricardi *et al.* 2016). The development of CRD, allowing risk determination for severe reactions and identifying the genuine allergen, has contributed to the improvement in diagnostic accuracy in food allergy.

In this review, we introduce buckwheat allergy and its causative allergens. The aim of this review was to explain the mechanisms or the factors in common buckwheat causing the allergic reaction and how the quality of life of patients and animals with this allergy can be improved.

Symptoms of buckwheat allergy

Buckwheat allergy is relatively infrequent—only 0.22% children of the school population in Japan have been reported to develop allergic reactions to common buckwheat (Takahashi *et al.* 1998). Nevertheless, common buckwheat is thought to be a serious food allergen because

it occasionally causes severe symptoms, such as anaphylaxis, in these patients (Imamura *et al.* 2008, Lee *et al.* 2016) (**Fig. 1**). The symptoms of buckwheat allergy include urticaria (Smith 1909), asthma (Stember 2006, Wieslander *et al.* 2000), atopic dermatitis (Chandrupatla *et al.* 2005, Wieslander *et al.* 2000), allergic rhinitis (Fritz and Gold 2003) and anaphylaxis (Schiffner *et al.* 2001). A case of food-dependent exercise-induced anaphylaxis caused by ingestion of common buckwheat has also been reported (Noma *et al.* 2001). Similar to baker's asthma, allergic reactions can be caused by common buckwheat inhalation (Obase *et al.* 2000).

Cases of buckwheat allergy have been commonly reported in Asian countries, such as Japan, Korea and China (Lee *et al.* 2016, Nakamura and Yamaguchi 1974, Wieslander *et al.* 2000). One reason for the high frequency of buckwheat allergy in these countries might be that people in Asian countries consume common buckwheat instead of staple foods as noodles, dumplings or porridges. In Japan and Korea, common buckwheat has to be specified in the food label as an ingredient because of the seriousness of the allergic reactions (Akiyama *et al.* 2011, Taylor and Baumert 2015, Teshima 2009).

Since the first case report of buckwheat allergy, at that time classified as 'buckwheat-poisoning' (Smith 1909), patients with allergy to common buckwheat have been reported in countries other than Asia (Asero *et al.* 2009, Badiu *et al.* 2013, Wang *et al.* 2006). In Japan, common buckwheat is consumed as 'soba' noodles (**Fig. 1**). However, in the western countries, common buckwheat is often mixed with certain dishes, for example, it is used as an agent along with pepper (Yuge *et al.* 2001) or is present in wheat burger (Wuthrich and Trojan 1995), porridges, pastas and pizzas (Heffler *et al.* 2007, 2011) as a hidden food allergen. Since there is no regulation against common buckwheat on the food labelling in any country other than Japan and Korea, foods possibly containing common buckwheat should be consumed with caution.

Furthermore, pillows filled with common buckwheat husks that are used in several Asian countries also cause allergic reactions (Hong *et al.* 1987, Matsumura *et al.* 1964, 1969, Rui *et al.* 2010). Several patients in Germany and the United States who were using pillows filled with common buckwheat husks also showed severe allergic reactions (Fritz and Gold 2003, van Ginkel 2002).

Celiac disease is a serious autoimmune disorder that causes symptomatic damage in the small intestinal villi. Since patients with celiac disease need to avoid gluten in foods, gluten-free foods have become widely prevalent (Saturni *et al.* 2010) and have become indispensable for people with gluten intolerance (Rai *et al.* 2018). In gluten-free foods, other grains, including common buckwheat, are used instead of wheat (Joshi *et al.* 2019). Common buckwheat contains functional ingredients and no gluten (Gimenez-Bastida and Zielinski 2015, Schoenlechner *et al.* 2010). Patients suspected with buckwheat allergy should be



Fig. 1. Illustration of animal feed and food containing typically common buckwheat and resulting mild to severe symptoms. Copyright by Fotolia.com: ©somma; ©dalaprod, ©Rock and Wask, ©tinglee1631, and by pixabay (<https://pixabay.com/de/>).

warned to check for common buckwheat as an ingredient while purchasing gluten-free food.

Food allergens

Buckwheat allergy presents an immediate hypersensitivity reaction that includes anaphylaxis mediated by a specific IgE antibody. It is therefore important to identify the buckwheat allergen molecules and clarify the mechanism of buckwheat allergy in order to develop an accurate diagnostic procedure and safe immunotherapy. The IgE-binding proteins causing allergy are called allergens or allergen components. A number of allergens have been identified and they are classified into few protein families with limited biochemical functions (Radauer *et al.* 2008). Most plant food allergens are classified into four protein superfamilies: prolamin superfamily, cupin superfamily, profilin and Bet v 1-related protein (Radauer and Breiteneder 2007, Satoh and Teshima 2016).

Several allergens have been named according to the allergen nomenclature (<http://www.allergen.org/>) (Pomes *et al.* 2018). In addition, the nucleotide sequences, deduced amino acid sequences, steric structures and binding sites with the IgE antibody present in the patients' sera called

IgE epitopes of some the identified allergens have been elucidated. There are several allergen databases with such information (**Table 1**). The characteristics of each database are different. For example, AllergenOnline is a useful allergen database for assessing novel food proteins with potential cross-reactivity (Goodman *et al.* 2016), while the Allergen Database for Food Safety contains information on the IgE-epitope sequence of each allergen (Nakamura *et al.* 2005).

To date, multiple allergen molecules have been found in causative foods, and a single causative food may have multiple allergen molecules. Moreover, the kind and amount of the causative allergens vary for each patient, and the allergen components are related to the type of symptoms. Therefore, measuring a specific IgE antibody titre for a single allergen molecule (allergen component) contributes to the improvement in the accuracy of the diagnosis (Valenta *et al.* 1999). The diagnosis using allergen components called as CRD has been used in recent years to improve the accuracy of allergy diagnosis. Therefore, the detection and identification of allergens can be important information for the diagnosis, prevention and treatment of allergens.

Table 1. Allergen databases

Name	URL
AllFam	http://www.meduniwien.ac.at/allergens/allfam/
Allergen Nomenclature	http://www.allergen.org
Allergome	http://www.allergome.org
Allergen Database for Food Safety (ADFS)	http://allergen.nihs.go.jp/ADFS/
Allergen Online (FARRP)	http://www.allergenonline.org/
Structural Database of Allergen Proteins (SDAP)	https://fermi.utmb.edu/
COMPARE Database	http://db.comparedatabase.org/

Table 2. Known buckwheat allergens

Name	Protein family/characteristics	MW (kDa)	References
Fag e 1	13S globulin	22	Urisu <i>et al.</i> (1994)
Fag e 2	2S albumin	17	Tanaka <i>et al.</i> (2002)
Fag e 3	7S globulin/vicilin	19	Park <i>et al.</i> (2000)
Fag e 4	Antimicrobial peptide	4	Geiselhart <i>et al.</i> (2018)
Fag e 5	Vicilin-like protein	55	Geiselhart <i>et al.</i> (2018)
Fag e 10 kDa	α -amylase inhibitor/trypsin inhibitor	16	Matsumoto <i>et al.</i> (2004)
Fag e TI	Trypsin inhibitor	9	Park <i>et al.</i> (1997)

Buckwheat allergens

Several buckwheat allergens have already been elucidated (Cho *et al.* 2015, Park *et al.* 2000, Urisu *et al.* 1994, Yoshimasu *et al.* 2000)—mostly seed storage proteins—and some of them have been registered in the databases (Table 2). An investigation of the binding activity with IgE in sera from allergic patients showed that Fag e 1 shows reactivity in all patients with buckwheat allergy, and it is considered to be a major allergen as it causes a reaction in >50% patients with buckwheat allergy (Urisu *et al.* 1994). Yoshioka *et al.* (2004) isolated the cDNA of Fag e 1 and identified the linear IgE epitopes in Fag e 1. A study combining 2D electrophoresis and western blot using patients' sera indicated the presence of novel 13S globulin protein subunits or isoforms (Satoh *et al.* 2011).

On the other hand, since Fag e 2 showed IgE reactivity using the sera of patients with history of anaphylactic reactions to common buckwheat, it is considered to be the causative allergen of anaphylaxis due to common buckwheat (Tanaka *et al.* 2002). Moreover, Fag e 2 was found to be resistant to pepsin digestion while Fag e 1 and Fag e 3 were digested quickly; thus, based on the available evidence, it can be considered that Fag e 2 is the causative allergen of buckwheat anaphylaxis. The full-length cDNA of Fag e 2 was cloned and it was found to be an allergen belonging to the 2S albumin family according to the deduced amino acid sequence (Koyano *et al.* 2006). The 2S albumin family also includes peanuts and castor bean allergens. In particular, peanut allergens Ara h 2 and Ara h 6 are considered to be the causative allergens of serious symptoms caused by peanut consumption (Kukkonen *et al.* 2015). A linear IgE-epitope of Fag e 2 and a critical amino acid residue in the

IgE-epitope for IgE-binding has been identified (Satoh *et al.* 2010). Fag e 10 kDa is an α -amylase inhibitor/trypsin inhibitor similar to Fag e 2 (Matsumoto *et al.* 2004). Fag e 3 is a vicilin-like seed storage protein and is one of the major allergens in common buckwheat (Choi *et al.* 2007, Park *et al.* 2000). Park *et al.* (2000) reported that Fag e 3 is a relatively specific allergen in patients with buckwheat allergy.

Varga *et al.* (2011) reported a case of anaphylaxis to common buckwheat in a 7-year-old boy and indicated that the 60–70-kDa 11S globulin in common buckwheat may induce anaphylaxis. This indicates the possibility of varieties of causative allergens for anaphylaxis.

Recently, Fag e 4 and Fag e 5 have been identified as buckwheat allergens as they reacted with the sera from patients with buckwheat allergy (Geiselhart *et al.* 2018). Fag e 4 is a hevein-like antimicrobial peptide, which comprises 40 amino acid residues and is potentially cross-reactive with latex. Fag e 5 has been identified as a partial peptide of vicilin-like protein, whose full-length cDNA has not been isolated.

Cross-reactivity

Few studies have shown cross-reactivity between buckwheat allergens and other allergens. Yamada *et al.* (1995) showed that IgE antibodies to common buckwheat were cross-reactive with that to rice. They showed the possibility that the IgE antibodies from the patients without any symptoms after common buckwheat ingestion might recognise the epitopes on buckwheat allergens, which cross-react with rice allergens, whereas IgE antibodies from the patients with severe symptoms after common buckwheat ingestion might bind to buckwheat-specific epitopes. This

indicates that the difference in IgE epitopes is related to the differences in the food allergens that react, and furthermore, the presence or absence of cross-reactivity.

We reported that the linear IgE-epitope of Fag e 2 has no known homologous epitopes except for one of the linear IgE-epitope of Fag e 1 (Sato *et al.* 2010). The increasing number of studies on IgE-epitope sequences from sera of patients with buckwheat allergy is expected to deepen the findings on cross-reactivity in common buckwheat.

Several case reports have shown the cross-reactivity between common buckwheat and latex (Abeck *et al.* 1994, De Maat-Bleeker and Stapel 1998), poppy seed (Oppel *et al.* 2006) and coconut (Cifuentes *et al.* 2015). In order to elucidate the cross-reactivity between common buckwheat and other foods, the allergens and their IgE epitopes that are targets of cross-reactivity need to be identified.

Diagnosis of buckwheat allergy

Since buckwheat allergy can lead to severe symptoms, it is important to choose the best methods/tests to make an accurate diagnosis. Yanagida *et al.* (2017) found that true allergic reactions to common buckwheat are rare among buckwheat-specific IgE-positive children; however, careful attention for possible anaphylactic reactions must be paid when performing the buckwheat OFC test. On the other hand, measuring specific IgE antibody titres to buckwheat allergen components in the diagnosis of buckwheat allergy is useful. Tohgi *et al.* (2011) indicated that the Fag e 2-specific IgE test is useful for the accurate diagnosis of buckwheat allergy, and native Fag e 2 purified from salt-soluble buckwheat protein is more valuable than recombinant Fag e 2. The importance of the four types of buckwheat allergen components in the diagnosis of buckwheat allergy has been assessed using recombinant allergen components expressed in *Escherichia coli*, and the measurement of Fag e 3-specific IgE antibody has been shown to improve the diagnostic accuracy (Maruyama *et al.* 2016). In addition, it has been reported that Fag e 3-specific IgE test can predict OFC results and OFC-induced anaphylaxis (Yanagida *et al.* 2018).

Buckwheat allergies in animals

Similar to humans, domestic animals, such as dogs, cats and horses, also develop IgE-mediated allergies (Gershwin 2015). Pollen (Jensen-Jarolim *et al.* 2015) and food (Pali-Scholl *et al.* 2017) are the major causative antigens of allergy in animals, but these causative allergens have not been investigated in detail. Horses that develop allergic reactions present with symptoms such as eczema, urticaria, chronic coughing or recurrent airway obstruction. Intradermal administration of an allergen extract is currently used for the diagnosis and treatment of allergic symptoms in animals. The recent development of CRD has made it possible to detect the causative allergens by using the sera of

patients. The allergen microarray ImmunoCAP® ISAC 112 (Phadia AB, Uppsala, Sweden) is a useful tool for detecting causative allergen components (Harwanegg and Hiller 2005, Hiller *et al.* 2002, Jakob *et al.* 2015). Because this test can be performed using collected and stored blood samples, it may be a very valuable tool for diagnostics. In a previous study, we customised ImmunoCAP® ISAC 112 for horses by adding 19 additional allergens and created ImmunoCAP ISAC 131 (Einhorn *et al.* 2018). Using ‘ImmunoCAP® ISAC 131’ we succeeded in detecting Fag e 2 as a causative allergen in 70% of investigated horses with allergic symptoms, likely sensitised by special ‘protein-rich’ horse diets, such as ‘horse muesli’ (Fig. 1). This indicates that CRD is also effective in identifying the sensitising allergens in affected animals.

Reducing the amount of buckwheat allergens in foods and production of hypoallergens

Since small amounts of common buckwheat causes serious allergic reactions, buckwheat allergen contents should be reduced in foods or eliminated from the diets. Therefore, there is much interest in reducing the allergenicity of common buckwheat and reducing each buckwheat allergen component. However, there are currently no reports of allergenic common buckwheat varieties with different allergenic properties.

The major buckwheat allergens seem to be mainly present in the embryo and not in the endosperm (Licen and Kreft 2005). The buckwheat embryo in common buckwheat seeds has a complicated structure, and it is difficult to completely remove the embryo when the buckwheat seeds are crushed to make buckwheat flour. Nevertheless, the distribution of buckwheat allergens differs within the seeds, and it is possible to make powders with different amounts of buckwheat allergens at the grinding stage (Morita *et al.* 2006).

Determination of the nucleotide/amino acid sequences, identification of IgE epitopes and prediction of the three-dimensional structures of allergens are also effective for the development of hypoallergenic molecules and safe immunotherapy. Mimotope screening has been used as a powerful tool for identifying epitopes, such as those on pollen (Jensen-Jarolim *et al.* 1998) as well as on food allergens (Lukschal *et al.* 2016). We identified a linear IgE-epitope on the food allergen Fag e 2 using a peptide array and mimotope screening but not conformational epitopes (Sato *et al.* 2010). On the other hand, the mutation of some of the cysteine residues affected the pepsin digestibility and IgE-binding activity (Sato *et al.* 2008). Moreover, Katayama *et al.* (2018) indicated that the phosphorylated Fag e 2 can induce stronger and safer desensitisation than intact Fag e 2.

Conclusion

Investigations on buckwheat allergy started later than

research studies on other food allergies, in spite of the severe allergic reactions reported by patients who consumed common buckwheat. Nevertheless, the recent progress in buckwheat allergy research is remarkable. A large-scale survey involving differences in countries, regions, ethnicities and eating habits for the analysis of reactivity of each buckwheat allergen in patients and their symptoms will contribute to the identification of causative buckwheat allergens and the establishment of an effective allergy test. In addition, finding an index like the probability curves shown in other food allergies may facilitate the accurate diagnosis of buckwheat allergy.

Author Contribution Statement

RS designed the manuscript. All authors drafted the manuscript. RS finalised the manuscript. All authors approved the final version of the manuscript.

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