

Measurement results of Mal d 1 protein in apples cultivated in Nagano prefecture, Japan

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Abstract

Pollen-food allergy syndrome (PFAS) associated with birch and alder pollinosis is becoming a problem in Japan, therefore, it is necessary to find apple cultivars in which Mal d 1, a major PFAS allergen component, is expressed at low levels. Relative quantification of Mal d 1 mRNA in 28 Japanese apple cultivars was performed, and several cultivars with low Mal d 1 expression were identified. As it is the protein and not mRNA that is the allergen, it was necessary to ascertain protein levels. To do this, an ELISA test was constructed for the quantification of Mal d 1 protein levels in apples cultivated in the Nagano prefecture. Mal d 1 extraction solutions were compared, and extraction efficiency was shown to be optimal with minimal sample variation when a composition of 1 mol/L sucrose, 2% polyvinylpyrrolidone (PVPP), 2 mmol/L EDTA, and 1 mmol/L diethyldithiocarbamic acid was used. Using this approach, Mal d 1 extraction and quantification were performed on 26 apple cultivars from Nagano prefecture. Of the cultivars tested, 24 were significantly lower in Mal d 1 protein than Sun fuji apples. These low Mal d 1 protein expression cultivars are proposed candidates for producing low-allergenic Japanese apples.

Keywords : Mal d 1, PFAS, ELISA, Japanese apple cultivar, PR-10

I Introduction

Pollen-food allergy syndrome (PFAS) is a class II food allergic condition in which patients, sensitized to pollen allergens, ingest fruits or vegetables that contain a triggering pan-allergen. When the pan-allergen is highly homologous to the pollen antigen, pollen antigen-specific IgE cross-reacts with the antigen in food, triggering an allergic reaction. Among food allergies, IgE-dependent immediate allergies that cause localized symptoms in the oral mucosa are called oral allergy syndrome (OAS), with most fruit and vegetable allergies said to be OAS^{1, 2)}. As most OAS causative allergens tend to lose their allergenicity after heat treatment or the action of digestive enzymes, the oropharyngeal symptoms tend to be mild unless a large quantity of the causative antigen is ingested³⁾. In addition, OAS forms the majority of PFAS cases in Japan, in which symptoms are localized to the oropharynx.

Regarding the relationships between pollen and food in PFAS, cross-reactivity between birch pollen and *Rosaceae* fruits, especially apples, has been reported in Northern Europe. In Japan, birch does not grow naturally except in areas such as the Hokkaido and Nagano prefectures^{4, 5)}. Birch does not grow in the flatlands of Honshu, yet PFAS cases still occur, therefore, pollen from other plants must be the cause. Alder pollen is reported as an important PFAS allergen in the Kanto region⁴⁾. Supporting this is the observation that the rate of *Rosaceae* fruit allergies in alder pollinosis patients is 16/19 cases (84%)⁴⁾.

PR-10, a plant pathogenesis-related protein (PR), is a pan-allergen that often causes cross-reactivity between plants as it is both ubiquitous in plants and has a high homology²⁾. IgE for Bet v 1, a PR-10 from birch pollen, causes apple PFAS due to similarities with Mal d 1, a PR-10 from apples. In addition, Aln g 1, an allergen component of alder pollen, is also a PR-10 with high homology to Bet v 1 and Mal d 1, and in the absence

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Abbreviations Used: CV, Coefficient of variation; EDTA, Ethylene diamine tetraacetate; ELISA, Enzyme-linked immunosorbent assay; OAS, Oral allergy syndrome; PFAS, Pollen food-allergy syndrome; PR-10, Pathogenesis-related protein-10; PVPP, Polyvinylpyrrolidone.