

# Proteomic and Allergenomic Profiling of Banana (*Musa* spp.): Identification of Potential Novel Allergens in Thai Adult Banana Allergy Cohort

Patcharaporn Sangsuwan<sup>1</sup>, Onrapak Reamtong<sup>2</sup>, Nitaya Indrawattana<sup>3-5</sup>, Nawannaporn Saelim<sup>3</sup>, Ratiporn Leeanan<sup>3</sup>, Thapani Srisai<sup>3</sup>, Chamard Wongsai<sup>3,6</sup>, Torpong Thongngarm<sup>3,6</sup>, Stephen Kwok-Wing Tsui<sup>7</sup>, Mongkhon Sompornrattanaphan<sup>3,6</sup>, Anchalee Tungtrongchitr<sup>3,5,8</sup>

<sup>1</sup>Graduate Program in Biodesign in Medicine, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand;

<sup>2</sup>Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; <sup>3</sup>Center of Research Excellence in Allergy and Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; <sup>4</sup>Biomedical Research Incubation Unit, Department of Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; <sup>5</sup>Biodesign in Medicine, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; <sup>6</sup>Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; <sup>7</sup>School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, People's Republic of China; <sup>8</sup>Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Correspondence: Mongkhon Sompornrattanaphan, Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, Email [mongkhon.som@mahidol.ac.th](mailto:mongkhon.som@mahidol.ac.th); Anchalee Tungtrongchitr, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, Email [anchalee.tun@mahidol.ac.th](mailto:anchalee.tun@mahidol.ac.th)

**Purpose:** Bananas are one of the most widely consumed fruits globally, particularly in Thailand. However, banana fruits have been recognized as allergenic, with the WHO/IUIS currently listing six identified banana allergens. This study investigates the proteome and allergenome of crude protein extracts from *Musa* 'Kluai Hom Thong' bananas.

**Patients and Methods:** Proteins were separated using two-dimensional gel electrophoresis (2DE) and characterized through 2DE-IgE immunoblotting with sera from 20 banana-allergic patients. Reactive protein spots were analyzed using LC-MS/MS, and proteins were identified via database searches against UniProt-Musaceae.

**Results:** A total of 559 proteins were identified, with 35 reactive protein spots detected across the sera samples, corresponding to 19 distinct proteins. Notably, none of these proteins have been previously reported as banana allergens, categorizing them as potential novel allergens. Among these, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and pathogenesis-related (PR) proteins are notable, as they are major allergens in other fruits and organisms.

**Conclusion:** Nineteen IgE-reactive proteins were identified from *Musa* 'Kluai Hom Thong', with GAPDH and PR proteins proposed as candidate novel allergens associated with systemic reactions and cross-reactivity. Future multi-center studies incorporating functional assays are needed to validate their clinical relevance and diagnostic potential.

**Keywords:** allergen identification, banana allergy, food allergens, IgE-reactive proteins, immunoproteomics, musaceae

## Introduction

Banana (*Musa* spp.) is among the most widely consumed fruits globally and is a dietary staple in tropical regions such as Asia, Latin America, and Africa. In Thailand, domestic banana consumption continues to rise in parallel with production, reflecting its central role in the national diet. Despite its nutritional value, banana has emerged as a clinically relevant allergenic food. The global prevalence of banana allergy ranges from 0.05% to 4%, depending on the setting and population studied.<sup>1</sup> Notably, a recent cohort from Thailand reported an exceptionally high proportion of anaphylaxis (87.9%) among adult patients with confirmed IgE-mediated banana allergy.<sup>2</sup> Diagnosis of banana allergy primarily relies on clinical history in conjunction with sensitization testing, such as skin prick test (SPT), prick-to-prick test (PTP), and serum-specific IgE (sIgE).<sup>3</sup> However, conventional tests often use crude allergen extracts, which contain a mixture of genuine allergens, cross-reactive carbohydrate

determinants (CCDs), and non-specific components. These mixtures may lack standardization and diagnostic precision, especially in populations exposed to diverse banana cultivars and aeroallergens.<sup>4</sup>

Banana allergens (e.g., Mus a 1–6) from *Musa acuminata* listed by the WHO/IUIS Allergen Nomenclature Sub-Committee, including profilin (Mus a 1), class I chitinase (Mus a 2), non-specific lipid transfer protein (LTP, Mus a 3), thaumatin-like protein (TLP, Mus a 4), beta-1,3-glucanase (Mus a 5), and ascorbate peroxidase (Mus a 6). Among these, Mus a 2, Mus a 4, Mus a 5, and Mus a 6 are recognized as major allergens. However, the other allergens causing banana allergy are still undiscovered.<sup>5,6</sup> Clinical data indicate that anaphylaxis is the predominant reaction (63%) among banana-allergic Thai adult patients following ingestion,<sup>2,7</sup> in apparent contrast to patterns observed in other regions.<sup>8–13</sup> This finding underscores the potential for distinct allergen sensitization profiles in this population.

To overcome these limitations, component-resolved diagnostics (CRD)—which detect IgE reactivity to individual, purified allergens—have been developed. CRD improves specificity by differentiating between primary sensitization and cross-reactivity and has shown clinical utility in multiple plant-food allergies. However, its application to banana allergy remains limited, particularly in Asian cohorts where cultivar-specific allergens may differ from those characterized in the West.<sup>14</sup> The identification of relevant major allergens in local banana cultivars is therefore a critical step toward developing CRD-based diagnostics and improving patient management in high-prevalence regions. Therefore, this study aims to investigate banana allergens specific to the Thai population, focusing on the popularly consumed banana cultivar *Musa* ‘Kluai Hom Thong’. We aim to achieve this by conducting a comprehensive analysis of the protein profile (proteome) and then identifying the allergenome—the allergens that trigger allergic reactions in Thai patients. To our knowledge, this is the first integrated analysis of this cultivar in an Asian cohort. The resulting population-relevant candidate allergens potentially provide a foundation for component-resolved diagnostics in the future.

## Materials and Methods

### Study Design and Participants

This cross-sectional case–control study was conducted at Siriraj Hospital, Bangkok, Thailand within the Thai Adult Banana Allergy Cohort (TABAC). Thirty adults were enrolled: 20 with allergist-confirmed banana allergy and 10 controls (five atopic, five non-atopic).

### Case Definition

Banana allergy required all of the following: (1) a compatible history of IgE-mediated reactions—oromucosal symptoms, urticaria, angioedema, or anaphylaxis—occurring within 3 hours of banana ingestion; (2) laboratory confirmation by either a positive prick-to-prick test (PTP) to fresh banana or banana-specific IgE  $\geq 0.35$  kUA/L (ImmunoCAP); and (3) a positive oral food challenge (OFC) with fresh banana (cultivar Kluai Hom Thong). When OFC was contraindicated or infeasible (e.g., severe anaphylaxis, serious comorbidities), reproducibility was required, defined as  $\geq 2$  distinct reactions within 12 months. Participants were subsequently invited to undergo an OFC with heat-processed banana (boiled at 120 °C for 15 minutes) to assess tolerability. Clinical history was obtained via allergist-administered structured interview using a prespecified case report form and corroborated by chart review.

### Controls

Atopic controls were defined by sensitization to  $\geq 1$  aeroallergen on skin prick testing with standardized extracts (ALK-Abelló A/S, Hørsholm, Denmark; positive if wheal  $\geq 3$  mm over saline control) and/or specific IgE  $\geq 0.35$  kUA/L, with no history of banana-related reactions. The aeroallergen panel included house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), American cockroach, German cockroach, Bermuda grass, Johnson grass, acacia, para grass, cat, and dog. Non-atopic controls had negative skin testing and negative specific IgE across the same panel and no history of atopy or banana allergy. All controls underwent an OFC with Kluai Hom Thong to confirm tolerance.

## Clinical Assessment and Serum Collection

All participants underwent skin prick testing with common aeroallergens and PTP with ripe *Musa* ‘Kluai Hom Thong’. Serum samples were obtained for banana-specific IgE measurement (ImmunoCAP, Thermo Fisher Scientific) and stored at  $-80^{\circ}\text{C}$  until used for immunoproteomic analyses.

## Protein Extraction and Profiling

The banana cultivar (*Musa* ‘Kluai Hom Thong’) was formally identified by botanist (Dr. Sunisa Sangvirodjanapat), Project of Institute Establishment for Sireeruckhachati Nature Learning Park. A voucher specimen (No. PBM 006361–006362) has been deposited in the Herbarium of Mahidol University, Project of Institute Establishment for Sireeruckhachati Nature Learning Park. Banana pulp was homogenized in lysis buffer, clarified, and dialyzed against phosphate-buffered saline. Protein patterns were assessed by SDS-PAGE under reducing conditions and visualized by Coomassie Brilliant Blue staining.

## Proteomic Analysis

Crude protein extracts were reduced, alkylated, digested with trypsin, and analyzed by nano-LC–MS/MS. Peptide spectra were searched against the UniProt–Musaceae database, and proteins were annotated using Gene Ontology (GO) molecular function terms. Relative protein abundance was estimated using the exponentially modified protein abundance index (emPAI), derived from the ratio of observed to theoretically observable peptides ( $\text{emPAI} = 10^{\text{PAI} - 1}$ ), which provides an approximate, label-free quantification within the same LC–MS/MS experiment. Complete peptide lists and detailed protein identification data are provided in Supplementary Material ([Supplementary Table 1](#)).

## Immunoproteomic Analysis

To identify IgE-reactive proteins, banana extracts were separated by two-dimensional electrophoresis (2-DE) and immunoblotted with sera from 20 allergic patients and 10 controls. Sera from both groups were processed under identical conditions to ensure consistent comparison. Bound IgE was detected with anti-human IgE and chemiluminescent substrate. Immunoreactive protein spots unique to allergic patients were excised from parallel gels, digested with trypsin, and analyzed by LC–MS/MS. Identified proteins were compared with known allergens reported in the WHO/IUIS database and relevant literature. Complete peptide lists and detailed protein identification data are provided in Supplementary Material ([Supplementary Table 2](#)).

## Data Analysis

Proteins were considered candidate allergens if consistently recognized by allergic sera but not by controls. IgE-binding frequency was expressed as the percentage of allergic patients recognizing each protein, as provided in Supplementary Material ([Supplementary Table 3](#)). This study was designed as a descriptive immunoproteomic investigation; therefore, no inferential statistical analyses were performed. Observational associations between IgE reactivity and clinical severity, such as recognition of GAPDH in patients with anaphylaxis, were noted but not statistically tested.

## Ethics

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 125/2024). Written informed consent was obtained from all participants prior to enrollment and sample collection.

## Results

### Participants

Thirty participants were enrolled: 20 adults with allergist-confirmed banana allergy and 10 controls (5 atopic, 5 non-atopic). Among allergic patients, 17/20 (85%) were female and the mean age was 35.6 years. The most frequent manifestations were oral allergy syndrome (19/20, 95%), respiratory symptoms (15/20, 75%), and gastrointestinal involvement (13/20, 65%). Systemic reactions were common, with anaphylaxis in 18/20 (90%), including one case of Kounis syndrome. Serum banana-specific IgE ranged from 0.96 to 17.0 kUA/L. No control participants reported allergic

symptoms or showed evidence of banana sensitization (Table 1). All controls were negative for latex sIgE by ImmunoCAP and reported no latex allergy. Among atopic controls, 2/5 demonstrated grass sensitization (one to Johnson grass, one to Para grass); other aeroallergen tests were negative.

## Proteomic Profiling of *Musa* ‘Kluai Hom Thong’

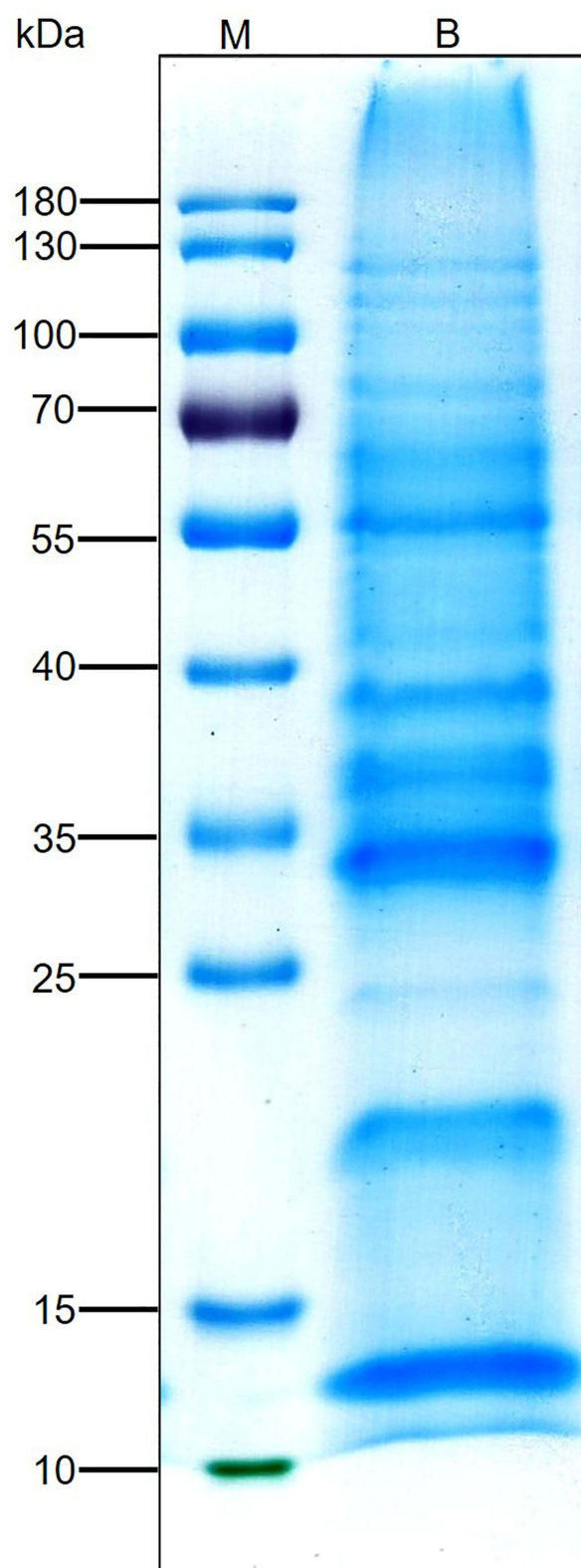
Proteomic profiling of *Musa* ‘Kluai Hom Thong’ revealed a wide distribution of proteins ranging from 10 to 180 kDa, with prominent bands at approximately 14, 20, 35, 40, and 55 kDa (Figure 1). Nano-LC–MS/MS identified 559 proteins, among which several previously reported banana allergens were detected, including class I chitinase (Mus a 2), non-specific lipid-transfer protein (Mus a 3) thaumatin-like protein (Mus a 4),  $\beta$ -1,3-glucanase (Mus a 5), and ascorbate peroxidase (Mus a 6). Relative abundance was estimated using the exponentially modified protein abundance index (emPAI), and the most abundant proteins are summarized in Table 2. The complete LC–MS/MS protein identification results are available in Supplementary Material (Supplementary Table 1).

**Table 1** Demographic Characteristics of 20 Banana-Allergic Patients and 10 Normal Controls

| No. | Sex    | Age | Symptom        | Severity                             | PTP to Banana | Banana Specific IgE (kUA/L) | Heat-Processed Banana Tolerance Status |
|-----|--------|-----|----------------|--------------------------------------|---------------|-----------------------------|----------------------------------------|
| P1  | Female | 41  | OAS, S, RS, GI | Anaphylaxis                          | +             | 1.04                        | Yes                                    |
| P2  | Female | 36  | OAS, S, RS, GI | Anaphylaxis                          | +             | 17.00                       | Yes                                    |
| P3  | Male   | 43  | OAS, S, RS     | Anaphylaxis                          | +             | 1.35                        | Yes                                    |
| P4  | Male   | 37  | OAS, S, RS     | Anaphylaxis                          | +             | 1.35                        | Yes                                    |
| P5  | Female | 29  | OAS, RS, GI    | Anaphylaxis                          | +             | 0.96                        | Yes                                    |
| P6  | Male   | 37  | RS, CVS        | Severe anaphylaxis (Kounis syndrome) | +             | 1.24                        | ND*                                    |
| P7  | Female | 27  | OAS, S, GI     | Anaphylaxis                          | +             | 11.10                       | Yes                                    |
| P8  | Female | 39  | OAS, GI        | Anaphylaxis                          | +             | 6.73                        | Yes                                    |
| P9  | Female | 33  | OAS, RS, GI    | Anaphylaxis                          | +             | 4.35                        | Yes                                    |
| P10 | Female | 44  | OAS, S, RS, GI | Anaphylaxis                          | +             | 3.93                        | Yes                                    |
| P11 | Female | 28  | OAS, S, RS     | Anaphylaxis                          | +             | 3.52                        | Yes                                    |
| P12 | Female | 28  | OAS, S, RS, GI | Anaphylaxis                          | +             | 3.36                        | Yes                                    |
| P13 | Female | 28  | OAS, S, RS, GI | Anaphylaxis                          | +             | 3.10                        | Yes                                    |
| P14 | Female | 34  | OAS, RS, GI    | Anaphylaxis                          | +             | 2.90                        | Yes                                    |
| P15 | Female | 49  | OAS, S, RS, GI | Anaphylaxis                          | +             | 2.74                        | Yes                                    |
| P16 | Female | 33  | OAS, S, RS, GI | Anaphylaxis                          | +             | 2.72                        | Yes                                    |
| P17 | Female | 41  | OAS, RS, CVS   | Anaphylaxis                          | +             | 2.54                        | Yes                                    |
| P18 | Female | 25  | OAS            | OAS                                  | +             | 2.30                        | Yes                                    |
| P19 | Female | 31  | OAS, S, GI     | Anaphylaxis                          | +             | 2.01                        | Yes                                    |
| P20 | Female | 48  | OAS            | OAS                                  | +             | 2.01                        | Yes                                    |
| N1  | Male   | 26  | Normal         | -                                    | -             | -                           | -                                      |
| N2  | Male   | 29  | Normal         | -                                    | -             | -                           | -                                      |
| N3  | Female | 25  | Normal         | -                                    | -             | -                           | -                                      |
| N4  | Female | 23  | Normal         | -                                    | -             | -                           | -                                      |
| N5  | Female | 30  | Normal         | -                                    | -             | -                           | -                                      |
| N6  | Female | 30  | Normal         | -                                    | -             | -                           | -                                      |
| N7  | Female | 52  | Normal         | -                                    | -             | -                           | -                                      |
| N8  | Female | 31  | Normal         | -                                    | -             | -                           | -                                      |
| N9  | Female | 26  | Normal         | -                                    | -             | -                           | -                                      |
| N10 | Female | 26  | Normal         | -                                    | -             | -                           | -                                      |

**Notes:** The level of sIgEs was determined using ImmunoCAP Allergen f92 ( $\geq 0.35$  kUA/L is considered positive). The Kluai Hom Thong banana was heat-treated by boiling at 120°C for 15 minutes.

**Abbreviations:** P, patient; N, normal; CVS, cardiovascular system; GI, gastrointestinal system; OAS, oral allergy symptoms; RS, respiratory system; S, skin; PTP, prick-to-prick test to banana; +, positive; -, negative; kUA/L, kilounits of allergen-specific IgE per liter; ND\*, not identify.



**Figure 1** SDS-PAGE separated banana *Musa* 'Kluai Hom Thong' stained with Coomassie Brilliant Blue G-250 dye (lane B). Lane M is a prestained broad-range protein standard. The number at the left is protein masses in kilodaltons.

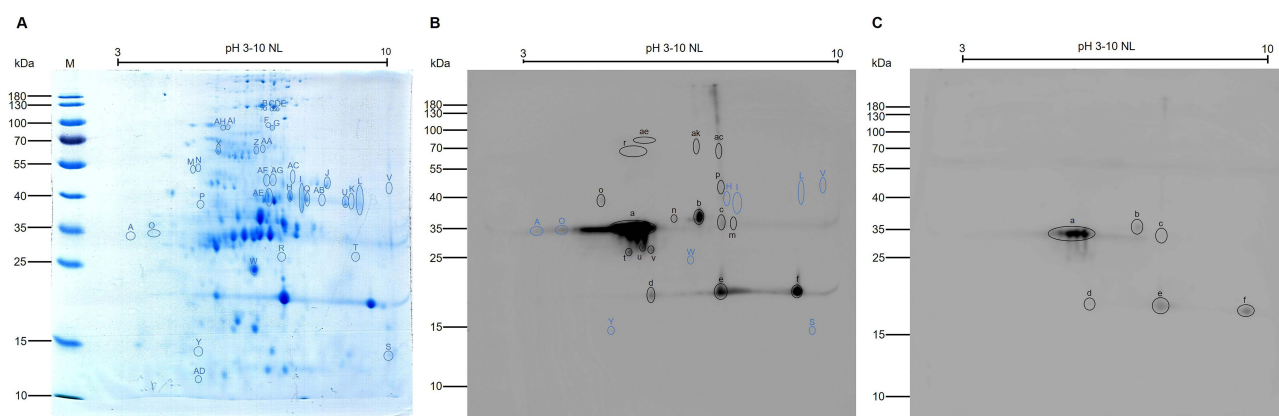
**Table 2** Top 20 Most Abundant Proteins in *Musa* 'Kluai Hom Thong' Based on emPAI Values

| Protein No. | Accession no. | Protein Identification                                                                    | Score | M.W. (Da) | No. of Peptide | Protein Cover | pI   | emPAI |
|-------------|---------------|-------------------------------------------------------------------------------------------|-------|-----------|----------------|---------------|------|-------|
| 1           | A0A1L2F3YI    | L-ascorbate peroxidase OS= <i>Musa acuminata</i>                                          | 385   | 27,406    | 15             | 76.3          | 5.01 | 4.84  |
| 2           | A0A804JN5I    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 1522  | 25,060    | 15             | 79.6          | 7.34 | 4.31  |
| 3           | A0A804I3E2    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 505   | 34,070    | 18             | 78.8          | 6.1  | 2.13  |
| 4           | E2J840        | Beta-1,3-glucanase I OS= <i>Musa</i> AB Group                                             | 400   | 33,903    | 15             | 52.8          | 8.96 | 1.86  |
| 5           | Q8VXF0        | Chitinase OS= <i>Musa acuminata</i>                                                       | 669   | 34,301    | 16             | 78.9          | 6.84 | 1.83  |
| 6           | A0A804JEC8    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 130   | 13,025    | 4              | 47.2          | 9.46 | 1.64  |
| 7           | A0A4S8JV38    | L-ascorbate peroxidase OS= <i>Musa balbisiana</i>                                         | 238   | 27,414    | 9              | 52.2          | 5.31 | 1.56  |
| 8           | Q2PWWI        | PME/invertase inhibitor-like protein (Fragment) OS= <i>Musa acuminata</i>                 | 225   | 20,593    | 10             | 52.3          | 5.67 | 1.54  |
| 9           | A0A804IH58    | Chitinase OS= <i>Musa acuminata</i> subsp. malaccensis                                    | 674   | 34,140    | 12             | 59.7          | 6.28 | 1.35  |
| 10          | A0A804J6V9    | Phosphopyruvate hydratase OS= <i>Musa acuminata</i> subsp. malaccensis                    | 256   | 48,255    | 19             | 63.1          | 5.67 | 1.25  |
| 11          | A0A804KIN8    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 229   | 36,398    | 12             | 45.7          | 7.02 | 1.23  |
| 12          | A0A804JC29    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 196   | 16,044    | 8              | 73.1          | 8.16 | 1.21  |
| 13          | A0A804L962    | Non-specific lipid-transfer protein OS= <i>Musa acuminata</i> subsp. malaccensis          | 90    | 12,225    | 4              | 33.3          | 8.84 | 1.17  |
| 14          | Q6QUK8        | Class III acidic chitinase OS= <i>Musa acuminata</i>                                      | 561   | 35,732    | 13             | 62.5          | 7.59 | 1.07  |
| 15          | A0A4S8KFJ8    | Thioredoxin domain-containing protein OS= <i>Musa balbisiana</i>                          | 106   | 13,018    | 6              | 63.2          | 5.32 | 1.07  |
| 16          | A0A804KYL6    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 224   | 17,626    | 12             | 63.4          | 6.17 | 1.06  |
| 17          | A0A804IXQ4    | (wild Malaysian banana) hypothetical protein OS= <i>Musa acuminata</i> subsp. malaccensis | 320   | 8771      | 3              | 37.7          | 8.92 | 1.03  |
| 18          | O22321        | Ripening-associated protein OS= <i>Musa acuminata</i>                                     | 161   | 14,554    | 4              | 44            | 6.82 | 0.92  |
| 19          | Q0Q296        | Pathogenesis-related protein I (Fragment) OS= <i>Musa acuminata</i>                       | 57    | 4525      | 1              | 65            | 7.96 | 0.9   |
| 20          | A0A4S8IFC0    | Jacalin-type lectin domain-containing protein OS= <i>Musa balbisiana</i>                  | 126   | 15,014    | 5              | 49.3          | 6.73 | 0.89  |

**Abbreviations:** emPAI, exponentially modified protein abundance index; pI, isoelectric point.

## Immunoproteomic Characterization of *Musa* 'Kluai Hom Thong'

Two-dimensional electrophoresis of banana protein extracts demonstrated distinct spot patterns (Figure 2A). Immunoblotting with sera from banana-allergic patients revealed multiple IgE-reactive spots (Figure 2B). Immunoblotting with serum from a normal control is shown in Figure 2C. Thirty-five IgE-reactive protein spots were



**Figure 2** 2D electrophoresis (2DE) of crude extract from *Musa* 'Kluai Hom Thong' and subsequent 2-DE-IgE immunoblotting. **(A)** The 2-DE gel was stained with Coomassie Brilliant Blue G-250; **(B)** The 2-DE-IgE immunoblot shows the IgE reactivity of serum from banana-allergic patient P14 with proteins in the banana extract. The patient's IgE recognized 9 distinct protein spots; **(C)** The 2-DE-IgE immunoblot shows the IgE reactivity of serum from a non-allergic control with the banana protein extract. The number at the left is protein masses in kilodaltons.

excised from Coomassie-stained gels and subjected to LC-MS/MS analysis. In total, 19 distinct proteins were identified. To facilitate clinical interpretation, these proteins were stratified into two categories (Table 3). High-relevance allergens, defined as those recognized by  $\geq 20\%$  of allergic sera or associated with systemic outcomes, included proteasome subunit beta (45%) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 40%), which was observed in patients with anaphylaxis and concomitant kiwi allergy, mechanosensitive ion channel protein (35%), and SCP domain-containing /pathogenesis-related (PR) proteins (20%). Exploratory allergens, recognized by fewer than 20% of sera without clear clinical correlation, included RNA polymerase II promoter Fmp27 domain (15%), alcohol dehydrogenase (10%), and leucine-rich repeat-containing proteins (10%).

Of particular note, GAPDH and PR proteins demonstrated not only frequent IgE recognition but also sequence homology to allergens in kiwifruit, rambutan, apple, and muskmelon, providing a plausible molecular basis for the cross-reactivity and systemic reactions reported in this cohort. IgE recognition of GAPDH was observed in patients with anaphylaxis, and PR proteins were also detected in individuals with systemic involvement. Although these associations are descriptive and not statistically tested, they highlight the potential clinical impact of these proteins. In contrast, several allergens previously described in banana, including Mus a 1–3 and Mus a 5, were not detected under our experimental conditions. Their absence is most likely due to abundance below the detection threshold rather than lack of expression, underscoring the importance of cultivar-specific allergen profiling.

**Table 3** Candidate Banana Allergens Identified by Immunoproteomics in Thai Patients with Banana Allergy

| Category              | Protein                                                       | % IgE Reactivity (n=20) | Clinical Correlation                                            |
|-----------------------|---------------------------------------------------------------|-------------------------|-----------------------------------------------------------------|
| <b>High relevance</b> | Proteasome subunit beta                                       | 45%                     | Frequently recognized; associated with systemic reactions       |
|                       | Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)              | 40%                     | Linked with anaphylaxis and concomitant kiwi allergy            |
|                       | Mechanosensitive ion channel protein                          | 35%                     | Detected in patients with systemic symptoms                     |
|                       | SCP domain-containing protein / PR proteins                   | 20%                     | Homology with known fruit allergens; potential cross-reactivity |
| <b>Exploratory</b>    | RNA polymerase II promoter Fmp27 domain                       | 15%                     | No clear clinical association                                   |
|                       | Alcohol dehydrogenase                                         | 10%                     | Limited recognition, no systemic correlation                    |
|                       | Leucine-rich repeat-containing proteins                       | 10%                     | No clear clinical relevance                                     |
|                       | Other proteins (eg, metabolic enzymes, hypothetical proteins) | <10%                    | Uncharacterized; requires further investigation                 |

In summary, proteomic and immunoproteomic characterization of *Musa* ‘Kluai Hom Thong’ identified 19 IgE-reactive proteins, with GAPDH and PR proteins emerging as candidate novel allergens of high clinical relevance. These findings provide insight into the molecular basis of banana allergy in Thai patients and may explain the high prevalence of systemic reactions and cross-reactivity. Figures and tables (Figures 1 and 2, Tables 1–3) support the prioritization of allergens according to clinical relevance and highlight promising targets for future diagnostic development.

## Discussion

This study provides the first immunoproteomic characterization of the banana cultivar *Musa* ‘Kluai Hom Thong’, which is the most widely consumed banana in Thailand, in a cohort of adults with clinically confirmed banana allergy. By integrating proteomic data with patient IgE profiles, we identified 19 IgE-reactive proteins, of which a subset demonstrated particular clinical relevance.

Four proteins were prioritized as high-relevance allergens: proteasome subunit beta (45% recognition), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 40% recognition, associated with anaphylaxis and concomitant kiwi allergy), mechanosensitive ion channel protein (35%), and SCP domain-containing/pathogenesis-related (PR) proteins (20%). The prominence of GAPDH and PR proteins is of particular interest, as GAPDH shares homology with allergens in kiwifruit and rambutan, while PR proteins share homology with allergens in kiwifruit, apple and muskmelon.<sup>15–18</sup> This provides a plausible molecular explanation for the frequent systemic reactions and cross-reactivity observed in Thai patients, highlighting their potential utility in component-resolved diagnostics.

Of note, GAPDH and PR proteins were frequently recognized. Although these associations were descriptive and not supported by statistical testing, they highlight the potential clinical impact of these proteins and reinforce their prioritization as candidate allergens for future validation.

Other identified proteins, including RNA polymerase II promoter Fmp27 domain, alcohol dehydrogenase, and leucine-rich repeat-containing proteins, were detected at lower frequencies (<20%) and without clear clinical correlation. While these exploratory candidates may represent additional allergens, further studies are needed to establish their clinical significance.

Our findings align with previous reports of Mus a 4 and Mus a 6 as abundant banana allergens but differ in that Mus a 1–3 and Mus a 5 were not detected in this study.<sup>19–21</sup> This absence is most likely due to abundance below the detection threshold of our proteomic approach rather than a true lack of expression. Such discrepancies underscore the influence of cultivar-specific and methodological factors on allergen profiling.<sup>22</sup>

These findings should be interpreted in light of study limitations. The small sample and single-center recruitment may restrict generalizability. Because this was an exploratory, descriptive study, we did not perform formal between-group comparisons of IgE frequencies. Functional validation was not undertaken (e.g., basophil activation, inhibition assays, or provocation with purified proteins); thus, the clinical allergenicity of identified proteins remains unconfirmed. Potential confounding by co-sensitization (e.g., pollens) may also have influenced IgE reactivity.<sup>7,23</sup> In this discovery cohort (n=20; ~90% anaphylaxis), per-patient IgE-binding profiles did not show a reproducible severity pattern: of the two non-anaphylaxis cases, P20 had no components distinct from anaphylaxis, and P18 exhibited seven singleton reactivities (each 1/20), which do not support severity inference. Accordingly, we do not claim a link between binding profiles and anaphylaxis; this will require larger, independent cohorts with prespecified endpoints and functional validation.

Future research should validate high-relevance allergens such as GAPDH and PR proteins in larger, multi-center cohorts and employ functional assays to confirm their clinical potency. Incorporation of these proteins into component-resolved diagnostic panels may enhance diagnostic accuracy, and investigation of their role in cross-reactive fruit and latex allergy syndromes could provide further insight into the molecular basis of systemic reactions.

## Conclusion

In summary, this study identified 19 IgE-reactive proteins from *Musa* ‘Kluai Hom Thong’, with GAPDH and PR proteins emerging as candidate novel allergens of high clinical relevance. These proteins may contribute to the frequent systemic reactions and cross-reactivity observed in Thai patients with banana allergy. While several exploratory proteins were also detected, their clinical significance remains uncertain. Importantly, the findings should be interpreted within the

limitations of this descriptive study, including the small sample size, single-center design, and absence of functional validation. Future studies in larger, multi-center cohorts, incorporating functional assays and statistical analyses, are warranted to confirm the clinical allergenicity of these proteins and support their potential inclusion in component-resolved diagnostic platforms.

## Ethic Approval

The study protocol was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (COA no. Si 125/2024). Laboratory analysis was conducted after IRB approval. Written informed consent was obtained from all participants prior to enrolment.

## Acknowledgments

We sincerely thank Ms. Aree Jameekornrak Taweethue, Ms. Orathai Theankeaw, Ms. Kitnittha Poladao, and Ms. Unchalee Ruangsirarak for their invaluable research assistance. We also extend our gratitude to Dr. Irin Vichara-anont, Dr. Settawut Taratikhundej, Dr. Thanachit Krikeerati, and Dr. Piyaporn Chokevittaya for their dedicated patient care, which was essential to the success of this study.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This research was supported by Mahidol University through the Fundamental Fund for the fiscal year 2024 (Grant Number FF-034/2567 and FF-133/2567) under the National Science Research and Innovation Fund (NSRF). Additional funding for the clinical component was provided by the Siriraj Research Development Fund, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Grant Number R016633029 and R016235025, the latter managed under the Routine to Research [R2R] program).

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Krikeerati T, Rodsaward P, Nawiboonwong J, Pinyopornpanish K, Phusawang S, Sompornrattanaphan M. Revisiting fruit allergy: prevalence across the globe, diagnosis, and current management. *Foods*. 2023;12(22):4083. doi:10.3390/foods12224083
2. Julianon N, Thiravetyan B, Unhapipatpong C, et al. Not just a banana: the extent of fruit cross-reactivity and reaction severity in adults with banana allergy. *Foods*. 2023;12(13):2456. doi:10.3390/foods12132456
3. Unhapipatpong C, Julianon N, Krikeerati T, Vichara-Anont I, Sompornrattanaphan M. Adult IgE-mediated food allergy is on the rise: a review of phenotypes, pathophysiologic mechanisms, diagnosis, and advances in management. *Asian Pac J Allergy Immunol*. 2022;40(4):308–320. doi:10.12932/AP-101122-1499
4. Dramburg S, Hilger C, Santos AF, et al. EAACI molecular allergology user's guide 2.0. *Pediatr Allergy Immunol*. 2023;34 Suppl 28:e13854. doi:10.1111/pai.13854
5. Delbourg MF, Guilloux L, Moneret-Vautrin DA, Ville G. Hypersensitivity to banana in latex-allergic patients. Identification of two major banana allergens of 33 and 37 kD. *Ann Allergy Asthma Immunol*. 1996;76(4):321–326. doi:10.1016/s1081-1206(10)60032-4
6. Nikolić J, Nešić A, Kull S, Schocker F, Jappe U, Gavrović-Jankulović M. Employment of proteomic and immunological based methods for the identification of catalase as novel allergen from banana. *J Proteomics*. 2018;175:87–94. doi:10.1016/j.jprot.2018.01.007
7. Thongkhom R, Oncham S, Sompornrattanaphan M, Laisuan W. Banana anaphylaxis in Thailand: case series. *Asia Pac Allergy*. 2020;10(1):e4. doi:10.5415/apallergy.2020.10.e4
8. Palacin A, Quirce S, Sanchez-Monge R, et al. Sensitization profiles to purified plant food allergens among pediatric patients with allergy to banana. *Pediatr Allergy Immunol*. 2011;22(2):186–195. doi:10.1111/j.1399-3038.2010.01125.x
9. Kocacik Uygün DF, Filiz S, Bingöl A. An evaluation of banana allergy in children living in the Mediterranean region. *Turk J Med Sci*. 2018;48(3):469–475. doi:10.3906/sag-1705-140

10. Grob M, Reindl J, Vieths S, Wuthrich B, Ballmer-Weber BK. Heterogeneity of banana allergy: characterization of allergens in banana-allergic patients. *Ann Allergy Asthma Immunol.* **2002**;89(5):513–516. doi:10.1016/S1081-1206(10)62090-X
11. Aytekin Guvenir F, Sengul Emeksiz Z, Buyuk Yaytokgil S, Toyran M, Dibek Misirlioglu E. Fruit allergy and anaphylaxis in children: culprit fruits and clinical findings. *Allergy Asthma Proc.* **2024**;45(4):e31–e37. doi:10.2500/aap.2024.45.240027
12. Şirin S, Özkan Kırın B, Özmen S, Akelma Z. Evaluation of allergic reactions and tolerance with fruit and vegetable allergy in children. *Int Arch Allergy Immunol.* **2024**;185(10):939–946. doi:10.1159/000539216
13. El-Sayed ZA, El-Ghoneimy DH, El-Shennawy D, Nasser MW. Evaluation of banana hypersensitivity among a group of atopic egyptian children: relation to parental/self reports. *Allergy Asthma Immunol Res.* **2013**;5(3):150–154. doi:10.4168/aa.2013.5.3.150
14. Riggioni C, Leung AS, Wai CY, et al. Exploring geographical variances in component-resolved diagnosis within the Asia-Pacific region. *Pediatr Allergy Immunol.* **2025**;36(3):e70054. doi:10.1111/pai.70054
15. Palacin A, Rodriguez J, Blanco C, et al. Immunoglobulin E recognition patterns to purified Kiwifruit (*Actinidia deliciosa*) allergens in patients sensitized to Kiwi with different clinical symptoms. *Clin Exp Allergy.* **2008**;38(7):1220–1228. doi:10.1111/j.1365-2222.2007.02927.x
16. Jirapongsananuruk O, Jirattanasopa N, Pongpruksa S, Vichyanond P, Piboonpocanun S. Glyceraldehyde-3-phosphate dehydrogenase as a major allergen in rambutan-induced anaphylaxis. *Ann Allergy Asthma Immunol.* **2011**;106(6):545–547. doi:10.1016/j.anai.2011.03.008
17. Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, et al. Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun.* **1995**;214(2):538–551. doi:10.1006/bbrc.1995.2320
18. Asensio T, Crespo JF, Sanchez-Monge R, et al. Novel plant pathogenesis-related protein family involved in food allergy. *J Allergy Clin Immunol.* **2004**;114(4):896–899. doi:10.1016/j.jaci.2004.06.014
19. Esteve C, D'Amato A, Marina ML, García MC, Righetti PG. In-depth proteomic analysis of banana (*Musa spp.*) fruit with combinatorial peptide ligand libraries. *Electrophoresis.* **2013**;34(2):207–214. doi:10.1002/elps.201200389
20. Peumans WJ, Proost P, Swennen RL, Van Damme EJ. The abundant class III chitinase homolog in young developing banana fruits behaves as a transient vegetative storage protein and most probably serves as an important supply of amino acids for the synthesis of ripening-associated proteins. *Plant Physiol.* **2002**;130(2):1063–1072. doi:10.1104/pp.006551
21. Clendennen SK, May GD. Differential gene expression in ripening banana fruit. *Plant Physiol.* **1997**;115(2):463–469. doi:10.1104/pp.115.2.463
22. Toledo T, Nogueira S, Cordenunsi B, et al. Proteomic analysis of banana fruit reveals proteins that are differentially accumulated during ripening. *Postharvest Biol Technol.* **2012**;70:51–58. doi:10.1016/j.postharvbio.2012.04.005
23. Reindl J, Rihs H-P, Scheurer S, Wangorsch A, Hausteiner D, Vieths S. IgE reactivity to profilin in pollen-sensitized subjects with adverse reactions to banana and pineapple. *Int Arch Allergy Immunol.* **2002**;128(2):105–114. doi:10.1159/000059400