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BBAP amplification profiles of apple varieties

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Original article

Abstract

Several types of allergies are currently known and are characterized by an exaggerated response of the immune system to substances from various sources called allergens. One of them is food allergy, which is becoming more common in the population. For this reason, it is necessary to describe the issue from several aspects including genomic variability of plant allergens. The objective of this study was to analyse intraspecific variability of Bet v 1 of 10 different varieties of apple species (*Malus domestica* Borkh.). BBAP technique for genomic determination of the presence of Bet v 1 homologs at the DNA level was performed. Degenerate primers that anneal a variable and conserved part of PR-10 protein homologues genes were used in the analyse. Amplicons were generated and formed relatively monomorphic profiles, indicating the stability of the given isoforms of Bet v 1 within the selected apple varieties. To evaluate the potential allergenicity of selected varieties further studies on another molecular level such as a comparison of gene expression of the PR-10 family members and their protein expression levels are needed.

Keywords

- Bet v 1
- allergens
- degenerate primers
- apple varieties

Authors contributions

A – Conceptualization
B – Methodology
C – Formal analysis
D – Software
E – Investigation
F – Data duration
G – Visualization
H – Writing – original draft preperation
I – Writing, reviewing & editing
J – Project administration
K – Funding acquisition

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Conflict of interest

None declared.

Introduction

The domesticated apple (*Malus domestica* Borkh.) belongs to the family *Rosaceae* and the genus *Malus* which includes 8–78 species [1]. Cultivation is widespread, especially in temperate areas, and there are estimated to be more than 10,000 varieties [2]. According to the Food and Agriculture Organization of the United Nations (FAO) at present time, China is the largest producer of domestic apple fruits [3] which are a rich source of phytochemicals including quercetin, catechin, phlorizin, and chlorogenic acid with strong antioxidant activity. Regular consumption of apples can reduce the risk of some cancers, cardiovascular disease, asthma, and diabetes [4].

In addition to the many health benefits that apples have, they can cause an allergic reaction in consumers. The main allergen of apples is the Mal d 1 protein, which belongs to group 10 of pathogenesis-related (PR) proteins. PR-10 proteins are 154 to 163 amino acids long with a molecular weight of approximately 17 kDa [5] and their expression is upregulated upon abiotic and biotic stress conditions [6]. Based on the similarity of the isoforms in the DNA sequences, the Mal d 1 proteins were divided into 4 main subgroups: Mal d 1.01, Mal d 1.02, Mal d 1.03, and Mal d 1.04 [7]. Since they are very similar, the binding capacity to IgE antibodies is not fundamentally different and the ability to elicit an immune response is probably determined by the degree of expression, not just the structure of the gene itself [8].

PR-10 is a variable group of proteins whose members can cross-react due to homology in conserved epitopes [9]. Conservation of epitope sites is demonstrated by cross-reaction, high variability in ypr10 genes *in silico* by comparison of individual known amino acid sequences of different plant species [10]. These two properties suggest the use of the gene as a DNA marker for the analysis of the variability of the ypr10 genes in plants. Marking techniques for generating polymorphisms amplifying the coding space of the genome are suitable for comparative mapping of gene pools [11].

Food allergies associated with birch pollen (*Betula*), which is the most dominant tree pollen in Northern and Central Europe [12], result from the initial sensitization to the major birch allergen (Bet v 1) and the subsequent immunological response of the IgE antibody specification to homologous proteins in food. One of the most important cross-reactions is the antibody response to apple consumption, which affects > 70% of patients allergic to birch pollen [13]. Cross-reactions originate from two antigens of different origins, which may have the same antigenic epitope that stimulates antibody production [14]. Often used term in connection with allergies is oral allergy syndrome (OAS) that defines a complex of symptoms induced by exposure of the oral and pharyngeal mucosa to food allergens. Some food Bet v 1-related proteins, lipid transfer proteins, and gibberellin regulated proteins involved in OAS are responsible for systemic reactions of patients from mild itching of the lips, mouth, and throat to lip and tongue swelling, to severe angioedema of the pharyngeal mucosa up to life-threatening emergencies [15]. The aim of this study was to analyse intraspecific variability of Bet v 1 sequences of 10 different varieties of apple species (*Malus domestica* Borkh.) using the BBAP technique.

Materials and methods

Plant material

Genomic analysis of intraspecific variability of domestic apple varieties (*Malus domestica* Borkh.), which are commercially available, were performed using a BBAP (Bet v 1-based amplicon polymorphism) technique. The set of analysed varieties consisted of the following ones: Spencer, Ecollete, Melrose, Jonalord, Angold, Sonet, Ligol, Kinova, Gala, and Pink Lady.

DNA isolation, quality, and quantity determination

Total genomic DNA was isolated using the GeneJETTM Plant Genomic DNA Purification Mini Kit from Thermo Scientific. The principle is based on the lysis cell membranes using a lysis solution, adding enzyme RNase to remove RNA and other solutions to wash the samples, followed by elution DNA from the column. Subsequently, the quality and quantity of the extracted DNA was determined spectrophotometrically using a NanoPhotometerTM (IMPLEN).

Nucleic acids display a maximum absorbance of UV light at 260 nm (A_{260}). Protein contamination in the samples was determined at a wavelength of 280 nm (A_{280}). Afterward, A_{260}/A_{280} ratios were calculated to indicate sample purity. The value $A_{260}/A_{280} = 1.8 \pm 0.1$ indicates relatively pure DNA [16]. Nucleic acid quantity was expressed in ng/µL.

PCR, electrophoresis, and analysis of results

Verification of PCR (polymerase chain reaction) functionality of the samples was performed using ITS (Internal Transcribed Spacer) sequences that are present in all Eukarya domain organisms. Primers for ITS sections (specifically ITS1 and ITS4) were designed by White et al. [17] and are commercially available. Positive amplification in PCR demonstrates the functionality and sufficient purity of the isolated nucleic acid, negatively excluding it. PCR products were separated on a 1.5% agarose gel and subsequently visualized using a UV transilluminator (BioDocAnalyze Box 2, Biometra, Germany).

The PCR reaction mixture consisted of MasterMix EliZyme HS Robust MIX, forward primer, reverse degenerated primer, and DNA from samples diluted 1:9. The PCR temperature regime began with input initiation at 95 °C for 5 minutes. This was followed by 40 cycles of denaturation at 95 °C, 45 s, deployment of primers at 54 °C, 45 s and polymerization at 72 °C, 35 s. Amplification was completed by final polymerization at 72 °C for 10 minutes. Bet v 1 degenerate PCR products were separated on a 10% polyacrylamide gel, read from the gel using the software GelAnalyzer 1.0, and transcribed into a binary matrix followed by construction of dendrogram using the UPGMA statistical method (http://genomes.urv.cat/UPGMA/) and Jaccard´s coefficient of genomic distances (Figure 2).

Databases and in silico analysis

All sequences in the work were searched using the NCBI bioinformatics database and compared using its own BLASTn algorithm. The searched sequences were checked for data from protein databases targeting protein allergens (Allergen Nomenclature).

In silico analyses for BBAP primer generation [18, 19] were performed using a megablast algorithm of available PR-10 protein sequences.

Design of degenerate primers

Degenerate primers were designed for Bet v 1 sequences with NCBI accession numbers: AJ289770.1 and AJ28977.1. The forward primer (F) is not degenerate, and its sequence is:
F: 5' CCT GGA ACC ATC AAG AAG 3'.
The reverse primer (R) has a degeneracy of two nucleotides and its sequence is:
R: 5' TTG GTG TGG TAS TKG CTG 3'.
Degeneracy of the primer is located at positions 12 (S) and 14 (K), whereas guanine or cytosine can occupy position 12, and thymine or guanine position 14. The reverse primer options are as follows:
R1: 5' TTG GTG TGG TAG TGG CTG 3'
R2: 5' TTG GTG TGG TAG TTG CTG 3'
R3: 5' TTG GTG TGG TAC TGG CTG 3'
R4: 5' TTG GTG TGG TAC TTG CTG 3' [19].

Results and discussion

Degenerate primer pair amplified a total of 3 amplicons (Figure 1). Based on BBAP profiles and the UPGMA method the samples were grouped into two clusters as is shown in the dendrogram (Figure 2). The sample profiles were very similar and formed 1 polymorphic and 2 monomorphic profiles (Table 1). The first group included varieties Spencer, Ecolette, Melrose, Jonalord, Angold, Sonet, and Kinova with an identical profile with amplicon lengths (195 bp and 400 bp). The second group included the varieties Ligol, Gala and Pink Lady with one additional amplicon of 705 bp in length. These three varieties come from the same generation of pedigree analysis. The result of the analysis is to obtain the genetic material from historic varieties, through ancestral and old, into recent and current ones. Each one of those varieties has a crucial position on a single breeding line and can be set in the generational scale. Information of their own breeding lines supports or questions their positions.

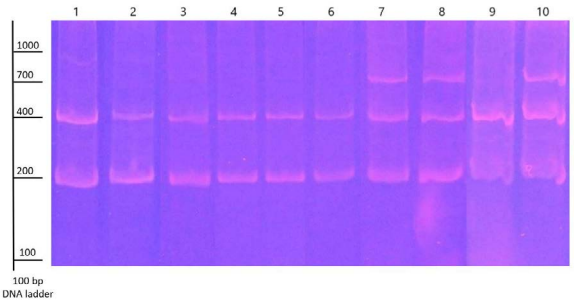

Figure 1. PCR amplicons with primer pair F + Rdeg on polyacrylamide gel

Table 1. Amplified fragments and polymorphism of 10 apple varieties

MW	Spencer	Ecollete	Melrose	Jon-alord	Angold	Sonet	Ligol	Kinova	Gala	Pink Lady
705	0	0	0	0	0	0	1	1	0	1
400	1	1	1	1	1	1	1	1	1	1
195	1	1	1	1	1	1	1	1	1	1

Figure 2. Dendrogram of BBAP amplification variability of genes of *Malus domestica* Borkh. varieties (primer combination F + Rdeg)

The five molecular marker techniques (Inter-Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP), Sequence-Specific Amplified Polymorphism (S-SAP), and Inter-Primer Binding Site (iPBS)) used in the study of Kuras et al. [20] detected DNA polymorphisms within all 15 apple genotypes studied (Golden Delicious, Gala, Jonagold, Šampion, and Idared and ten of their sports). The SSR and ISSR markers were found to be useful for cultivar identification and assessment of phenetic relationships, revealing advantages, due to higher reproducibility, over other commonly employed PCR-based markers such as RAPD or AFLP [20, 21]. CDDP (Conserved DNA-Derived Polymorphism) marker technique was used to analyse polymorphism within the genomes of 15 apples (Ambrosia, Gala, Gloster, Granny Smith, Jonaprince, May Gold, Melodie, Modi, Paula Red, Pink Lady, Pinova, Red Delicious, Renoire, Sampion and Selena) using five primer pair combinations. It was proved that CDDP markers are a suitable tool to produce polymorphic amplification patterns in apple genotypes [22].

Genetic markers are not influenced by physiology or environment, so they have many advantages compared to biochemical markers. They can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related cultivars. To assess the genetic diversity in plant species, exist many different types of molecular markers but no single technique is universally ideal. Therefore, the choice of the technique depends on the objective of the study, skills, financial or technical possibilities [23, 24].

Asero et al. [25] marked that exists inter-apple and intra-apple variability. Consequently, the amount of Mal d 1 in apples classified as containing low concentrations of allergen may be sufficient to induce both clinical symptoms and skin reactivity in birch pollen-allergic patients. Differences in the concentration of Mal d 1 proteins year-on-year or even between two growing sites are also confirmed [26], which suggests that external conditions can significantly affect the final concentration of Mal d 1 and thus the allergenicity of the fruit consumed. It was found that enzymatic browning led to a strong decrease of Mal d 1 and was significantly correlated with a decrease in total polyphenols [27].

The assessment of allergen identification, variability of allergenic molecules, and genetic relationships is important not only for the scientific community but also for consumers as the number of allergy sufferers increases every year, which results in an impact on the quality of their life. The comparison of generated Bet v 1 profiles of analysed apple varies show two main clusters. They differ in the amplification pattern of Bet v 1 homologs, which were matched by degenerate primers. To be able to link this information with their potential allergenicity, further comparison of the members of these groups in the PR-10 gene expression, as well as protein expression in these varieties, is needed.

Conclusions

Designed primers for the Bet v 1 allergen gene, which are degenerate, can capture not only different isoforms of the Bet v 1 gene but also its homologues. Bet v 1-based amplicon polymorphism technique was used to analyse intraspecific variability of 10 apple varieties. In our study, we observed a relatively low level of polymorphism in amplification patterns that indicates the stability of the given isoforms within the selected apple varieties.

References

[1] Phipps JB, Robertson KR, Smith PG, Rohrer JR. A checklist of the subfamily Maloideae (Rosaceae). Canadian Journal of Botany. 1990;68(10):2209–2269. doi: https://doi.org/10.1139/b90-288.

[2] Janick J, Moore JN, editors. Fruit breeding, tree and tropical fruits. New York: John Wiley & Sons; 1996. p. 1–77.

[3] Food and Agriculture Organization of the United Nations (FAO). [Internet] 2019. Available from: http://www.fao.org.

[4] Boyer J, Liu RH. Apple phytochemicals and their health benefits. Nutrition Journal. 2004;3:5. doi: https://doi.org/10.1186/1475-2891-3-5.

[5] Somssich IE, Schmelzer E, Kawalleck P, Hahlbrock K. Gene structure and in situ transcript localization of pathogenesis-related protein 1 in parsley. Molecular and General Genetics. 1988;213:93–98. doi: https://doi.org/10.1007/BF00333403.

[6] Fernandes H, Michalska K, Sikorski M, Jaskolski M. Structural and functional aspects of PR-10 proteins. The FEBS Journal. 2013;280:1169–1199. doi: https://doi.org/10.1111/febs.12114.

[7] Matthes A, Schmitz-Eiberger M. Apple (Malus domestica L. Borkh.) allergen Mal d 1: effect of cultivar, cultivation system, and storage conditions. Journal of Agricultural and Food Chemistry. 2009;57:10548–10553. doi: https://doi.org/10.1021/jf901938q.

[8] Son DY, Scheurer S, Hoffmann A, Haustein D, Vieths S. Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. European Journal of Nutrition. 1999;38:201–215. doi: https://doi.org/10.1007/s003940050063.

[9] Moraes AH, Asam C, Almeida FCL, Wallner M, Ferreira F, Valente AP. Structural basis for cross-reactivity and conformation fluctuation of the major beech pollen allergen Fag s 1. Scientific Reports. 2018;8(1):1–10. doi: https://doi.org/10.1038/s41598-018-28358-1.

[10] Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. The Journal of Allergy and Clinical Immunology. 2000;106(1):27–36. doi: https://doi.org/10.1067/mai.2000.106929.

[11] Varshney RK, Graner A, Sorrells ME. Genic molecular markers in plants: development and applications. Genomics-Assisted Crop Improvement: vol. 1: Genomics Approaches and Platforms. Dordrecht: Springer; 2007. p. 13–29.

[12] Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. Allergy. 2019; 74:1237–1248. doi: https://doi.org/10.1016/j.tibtech.2004.11.005.

[13] Ballmer-Weber BK. Food allergy in adolescence and adulthood. Chemical Immunology and Allergy. 2015;101:51–58. doi: https://doi.org/10.1159/000371669.

[14] Fu L, Cherayil BJ, Shi H, Wang Y, Zhu Y. Species and structure of food allergens: epitopes and cross-reactivity. In: Food Allergy: From Molecular Mechanisms to Control Strategies. Singapore: Springer; 2019. p. 13–39.

[15] Pastorello EA, Ortolani C. Oral allergy syndrome. In: Metcalfe DD, Sampson HA, Simon RA, editors. Food Allergy: Adverse Reactions to Food and Food Additives. Cambridge: Blackwell Science; 1997. p. 221–234.

[16] Desjardins P, Conklin D. Nanodrop microvolume quantitation of nucleic acids. Journal of Visualised Experiments. 2010;45:2565. doi: https://doi.org/10.3791/2565.

[17] White TJ et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols a guide to methods and applications. San Diego: Academic Press; 1990. p. 315–322.

[18] Žiarovská J, Zeleňáková L. Central and Eastern European spring pollen allergens and their expression analysis-state of the art. Diversity. 2016;8(4):1–11. doi: https://doi.org/10.3390/d8040019.

[19] Urbanová L, Žiarovská J. Variability of DNA based amplicon profiles generated by Bet v 1 homologous among different vegetable species. Acta Fytotechnica et Zootechnica. 2021;24(1):1–6. doi: https://doi.org/10.15414/afz.2021.24.mi-apa.1-6.

[20] Kuras A, Antonius K, Kalendar R, Kruczynska D, Korbin M. Application of five DNA marker techniques to distinguish between five apple (Malus × domestica Borkh.) cultivars and their sports. The Journal of Horticultural Science and Biotechnology. 2013;88(6):790–794. doi: https://doi.org/10.1080/14620316.2013.11513040.

[21] Goulao L, Oliveira CM. Molecular characterisation of cultivars of apple (Malus × domestica Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica. 2001;122:81–89. doi: https://doi.org/10.1023/A:1012691814643.

[22] Bilčíková J, Farkasová S, Žiarovská J. Genetic variability of commercially important apple varieties (Malus × domestica Borkh.) assessed by CDDP markers. Acta Fytotechnica et Zootechnica. 2021;24:21–26. doi: https://doi.org/10.15414/afz.2021.24.mi-apa.21-26.

[23] Kafkas S, Özgen M, Doğan Y, Özcan B, Ercişli S, Serçe S. Molecular characterization of mulberry accessions in Turkey by AFLP markers. Journal of the American Society for Horticultural Science. 2008;4:593–597. doi: https://doi.org/10.21273/JASHS.133.4.593,

[24] Pavlović N, Zdravković J, Cvikić D, Zdravković M, Adžić S, Pavlović S, Šurlan-Momirović G. Characterization of onion genotypes by use of RAPD markers. Genetika. 2012;2:269–278. doi: https://doi.org/10.2298/GENSR1202269P.

[25] Asero R, Marzban G, Martinelli A, Zaccarini M, Laimer da Camara Machado M. Search for low allergenic apple cultivars for birch pollen allergic patients: is there a correlation between in vitro assays and patient response? European Annals of Allergy and Clinical Immunolgy. 2006;38(3):94–98.

[26] Sancho AI, Foxall R, Browne T, Dey R, Zuidmeer L, Marzban G, Waldron KW, van Ree R, Hoffmann-Sommergruber K, Laimer M, Mills EN. Effect of postharvest storage on the expression of the apple allergen Mal d 1. Journal of Agricultural and Food Chemistry. 2006;54(16):5917–23. doi: https://doi.org/10.1021/jf060880m.

[27] Kschonsek J, Dietz A, Wiegand C, Hipler UCh, Böhm V. Allergenicity of apple allergen Mal d 1 as effected by polyphenols and polyphenol oxidase due to enzymatic browning. LWT. 2019;113:108289. doi: https://doi.org/10.1016/j.lwt.2019.108289.