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Polymorphism of Bolivian accessions of *Arachis hypogaea* L. revealed by allergen coding DNA markers

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Abstract: *Arachis hypogaea* L. is an annual legume that is one of the most consumed plant species. On the other hand, it belongs to one of the most monitored clinically important allergens worldwide. The polymorphism of this species based on allergen coding genes could be useful in its characterisation, but previously, no allergen-based marker techniques have been developed for peanuts. A new type of DNA-based markers of coding regions were used to analyse the variability of 21 peanut accessions – BBAP (Bet v1 based amplicon polymorphism), PBAP (profilin based amplicon polymorphism), and VBAP (vicilin based amplicon polymorphism). All of the used technique provided polymorphic fingerprints and distinguished the analysed peanut accessions. The effectivity of these techniques corresponds to the presence of the allergen homologous sequences that are a part of the *A. hypogaea* genome. VBAP was the most effective in distinguishing the analysed peanut accessions when compared to the results of BBAP and PBAB. For BBAP, two of the analysed accessions provided the same fingerprinting pattern. The ability of the used markers to detect polymorphisms was comparable, with an average polymorphism information content (PIC) value of 0.47.

Keywords: allergen markers; DNA analysis; food safety; genetic resources; peanut accessions

The genus *Arachis* from the family Fabaceae consists of numerous described species that have been assembled into nine taxonomic sections based on morphological characters, geographic origin, and cross-compatibility (Krapovickas et al. 2007). The most known species of this genus, *A. hypogaea,* is a valuable oil seed crop primarily grown in semiarid tropical, subtropical, and warm temperate regions of nearly 100 countries in six continents between 40N and S of the equator (Proite et al. 2007,

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FAO-ALENU 2020). *Arachis hypogaea* has its origin in southern Bolivia/northwestern Argentina (Dillehay et al. 2007). A very variable range of distinctive environments exists in these regions, predominantly in the Andes' eastern foothills, that provide diverse stress and adaptation conditions, causing polymorphism in the peanut genome. *Arachis hypogaea* is an allotetraploid species ($2n = 4x = 40$, AABB) with a relatively large and complex genome.

Because of the crucial economic importance of peanuts, their germplasm is characterised and maintained worldwide in different *ex-situ* collections (Benz 2012, Williams et al. 2022). DNA markers are applied widely to characterise peanut genetic resources and analyse different questions related to its genomic variability or for the purposes of molecular breeding.

Previously, microsatellite or simple sequence repeat (SSR) markers have been widely used to reveal cultivated peanuts' variability and cross-transferability by several studies (Gimenes et al. 2007, Luo et al. 2017). SSR markers were concluded to be useful for peanut germplasm analysis, diversity studies, linkage mapping, and phylogenetic relationships (Cuc et al. 2008, Hong et al. 2021). Two integrated consensus genetic maps with markers, such as EST-SSRs, transcriptome-SSRs, and g-SSRs, were constructed for cultivated peanut and wild relatives (Shirasawa et al. 2013, Lu et al. 2018). Additionally, a highly informative set of SSR markers was used to screen parental accessions of peanut mapping populations for molecular breeding (Pandey et al. 2012) and SSR markers and intron sequences were used to study phylogenetic relationships of cultivated peanut and wild species of *Arachis* (Moretzsohn et al. 2013).

Random amplified polymorphism detection fingerprints were defined in the genome of *A. hypogaea* (Raina et al. 2001, Sai et al. 2016) and used to link some important resistance genes (Mondal et al. 2007). Retrotransposon-based markers were applied to analyse peanut germplasm by inter-primer binding sites polymorphism (Montero-Torrez et al. 2020) or by a combination of retrotransposon markers with cleaved amplified polymorphic sequences (CAPS) markers (Gayathri et al. 2018).

Advances in plant genomes sequencing projects and the rising amount of known sequences stored in public databases have led to the development of diverse DNA markers focused on mapping different types of polymorphism. DNA markers from the different coding regions of plants' genomes can provide specific information on genomic polymorphism. For instance, start codon targeted markers have been applied for genetic diversity analysis of different plant species, such as chickpeas (Pakseresht et al. 2013), potatoes (Gorji et al. 2011), tomatoes (Shahlaei et al. 2014), castor (Vivodík et al. 2019), and wheat (Seyedimoradi et al. 2016). In the case of *A. hypogaea*, start codon targeted (SCoT) markers were used for the purposes of functional genetic variation mapping (Xiong et al. 2011), and this technique provided a level of polymorphism that ranged from 14.29% to 66.67%, and a high genetic similarity was obtained for the analysed peanut accessions. P450 based analogue (PBA) markers have been applied for genetic diversity analysis for plant species such as mango, ginger (Jatoi et al. 2010), and ivy (Bošeľová et al. 2016).

Arachis hypogaea belongs among the top fifteen most important feeding crops and the second most important legume crop in global production (FAOSTAT 2021). Regarding its allergen potential, peanuts are one of the frequent causes of acute food allergies, one of the eight major food allergens with a worldwide prevalence of up to 2%, and are on the rise (Hilu et al. 2019). The pattern of sensitisation to peanut allergens varies among populations in different geographical regions (Vereda et al. 2011). The Ara h 1, Ara h 2, and Ara h 3 from peanuts are the major allergens in the USA and are often associated with severe symptoms. Spanish patients are more often sensitive to the Ara h 9. Swedish patients have the highest sensitisation rate to Ara h 8, a cross-reactive homologue of the birch pollen allergen Bet v 1. In screening of peanut-allergic patients from 11 European countries, Ara h 2 was identified as the major allergen (Ballmer-Weber et al. 2015). Geographical differences were observed between Ara h 8 and Ara h 9, which are major allergens for Central/Western and Southern Europeans, respectively. In a study of peanut-allergic patients from the Netherlands, the most frequently recognised allergen was also Ara h 2 (Koppelman et al. 2004).

Allergen coding gene variability is well studied across various plant species (Sinha et al. 2014, Führer et al. 2021), providing a good source of PCR-based markers. Although diverse types of molecular markers have been utilised in peanuts, no molecular marker targeting the allergen coding regions has been developed, highlighting a crucial research gap. DNA markers from allergen coding regions will not only allow for better characterisation of accessions based on allergens but still, but they will also allow breed-

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Table 1. List of *Arachis hypogaea* accessions

No.	Accession	Common name	Collection place	No.	Accession	Common name	Collection place
1	CMC-003	Overo pecho blanco	Central Chuquisaca	12	CMC-006	Osco	Central Chuquisaca
2	CMC-008	Chaucha roja		13	CMC-019	Oclliri colorado	
3	CMC-004	Fusilero		14	CMC-015	Cormino	
4	CMC-005	Overo grande		15	CMC-002	Phiti cintura colorado	
5	CMC-016	Guano de oveja		16	CMC-009	Blanco quinsancho	
6	CMC-018	Yungueño		17	CMC-017	Phiti cintura ladrillo	
7	CMC-010	Larguillo		18	T1	*Wild plant	Chuquisaca Chaco
8	JJV-002	Overo		19	T5AN1890	*Wild plant	Chuquisaca Chaco
9	CMC-012	Caspeado		20	CMC-013	Chaucha blanca	Central Chuquisaca
10	CMC-014	Pico loro		21	PL-ICLA	Pico loro	North Chuquisaca
11	CMC-001	Maní blanco		–	–		

ers to screen accessions based on allergen types for breeding purposes. Hence, the development of these types of markers, especially in allergen-prone crops like peanuts, is crucial from a breeding point of view.

In the current study, DNA-based markers of allergen coding regions were applied to analyse the polymorphism of peanut germplasm, which has not been used before for this species. The markers included Bet v 1-based amplicon polymorphism (BBAP), profilin-based amplicon polymorphism (PBAP), and vicilin-based amplicon polymorphism (VBAP). The BBAP method exploits widespread Bet v 1 gene homologs in plant genomes, validated across species (Žiarovská and Zeleňáková 2018). Ara h 8, akin to birch pollen allergen Bet v 1, triggers peanut allergy (Hulburt et al. 2013). PBAP leverages prevalent profilin gene homologs, including Ara h 5, linked to pollen-induced peanut allergy (Shewry et al. 1995). VBAP targets legume genomes, amplifying allergenic vicilins like Ara h 1 and Ara h 3 (Viquez et al. 2003). These allergens, thermally stable, play a pivotal role in peanut hypersensitivity (Mueller et al. 2014). PBAP and VBAP were used for the analysis of *A. hypogaea* for the first time. These markers are universal in their use in plants as they are mapped from the homologs of allergen coding genes, and have been used to study polymorphism variability among various plants (Žiarovská and Urbanová 2022).

The findings shed light on the intricate allergenic profiles of peanuts from Bolivia and expanded our understanding of cross-reactivity among allergens. These methodologies not only provide insights into allergen diversity and prevalence but also offer valuable tools for targeted allergen detection and potential mitigation strategies. By unravelling the genetic underpinnings of allergens in peanuts, these findings contribute to advancing personalised allergy management and enhancing food safety measures for individuals susceptible to peanut-related allergies.

MATERIAL AND METHODS

Plant material. The polymorphism analysis was carried out in the peanut collection, which consisted of 21 accessions, from the Germplasm Bank of the Institute of Biodiversity and Natural Resources (BIORENA), University of Saint Francis Xavier, Bolivia (Table 1). The accessions were collected in Chuquisaca Department (Latitude: 20°00'00"S, Longitude: 64°25'00"W) in Bolivia, specifically in the Municipal Associations of Central Chuquisaca, north Chuquisaca and Chuquisaca Chaco (Figure 1). These are cultivars grown by local farmers, except for

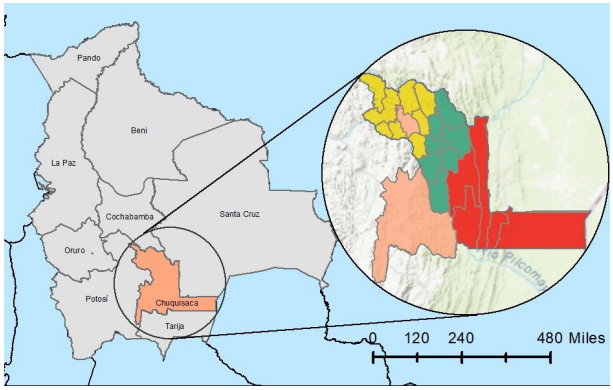

Figure 1. Chuquisaca Department in Bolivia from where the accessions were collected: Municipal Associations of Central Chuquisaca (green colour), north Chuquisaca (yellow colour) and Chuquisaca Chaco (red colour)

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accessions 18 and 19, which are wild peanut plants. For the analyses, seeds were collected from the agricultural campaign 2018 (March) after 160 days of cultivation. The seeds were planted in pots under greenhouse conditions at the Faculty of Tropical AgriSciences, Czech University of Life Sciences in Prague, Czech Republic. Subsequently, the young seedlings (two weeks old) were transported in pots to the AgroBioTech Research Centre, the Slovak University of Agriculture in Nitra, Slovak Republic, for further analysis. Ten young green leaves from individual plants were used for the extraction of mixed genomic DNA.

Genomic DNA extraction. GeneJET Plant Genomic DNA Purification Kit (ThermoFisher Scientific, Waltham, USA) was used to extract DNA from the samples, and the supplier's instructions were followed without modification. The quantity and quality of the DNA extracted was assessed spectrophotometrically by nanophotometer P360 (IMPLEN, GmBH, Munchen, Germany).

A control PCR reaction with ITS (internal transcribed spacer) primers was performed to ensure the functionality of DNA in PCR reactions. In the control PCR, ITS1 (forward) and ITS 4 (reverse) primers were used, according to White et al. (1990). The PCR conditions for functionality tests were as follows: initial denaturation at 95 °C, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, final extension was held for 15 min at 72 °C.

PCR amplification and data analysis. Degenerate primers were used according to a previously reported study for BBAP (Žiarovská and Zeleňáková 2018) and PBAP (Klongová et al. 2021), and VBAP analysis. The sequences of primers were checked in silico to match the peanut allergen sequences with the positive match for *A. hypogaea* Ara h 8 allergen mRNA, complete cds (AY328088.1) as well as for *Arachis hypogaea* profilin (Ara h 5) mRNA, complete cds (AF059616.1). No nucleotide sequence is actually reported in public databases for peanut vicilin.

The PCR reactions were performed on a professional Basic gradient XL (BIOMETRA, Jena, Germany) thermocycler. All PCR reactions were performed in 10 µL volume; primers were added in 500 nmol concentrations for BBAP and 400 nmol concentrations, respectively, for PBAP and VBAP, based on the results of the optimisation procedure. DNA at a normalised concentration of 50 ng/µL was added with MasterMix Robust HS Elizyme (ELISABETH PHARMACON, Brno, Czech Republic). The thermal profile followed the steps: initial denaturation at 95 °C followed by 40 cycles of denaturation at 95 °C for 45 s, annealing at 54 °C for 45 s and elongation at 72 °C for 35 s; final elongation was held at 72 °C for 10 min (Žiarovská et al. 2021).

The PCR fragments were separated on a 2% agarose gels stained with GelRed® Nucleic Acid Gel Stain (BIOTIUM, Fremont, USA) and visualised by UV-transilluminator-BDAdigital system 30 (Analytik Jena, Jena, Germany). According to the amplicons, binary matrixes were created with the help of the free online software Gelanalyzer (www.gelanalyzer.com).

The polymorphism information content (PIC) was calculated by using the PIC = 1 – [f^2 + (1 – f)^2] formula, where f is the marker frequency in the data set (Roldán-Ruiz et al. 2000, De Riek et al. 2001). The percentage of polymorphism was based on directly counting polymorphic and total loci. Marker index (MI) was calculated based on polymorphic information index and effective multiplex ratio: MI = PIC × $(n_p(n_p/n))$, where n_p is the number of polymorphic loci, and n is the total loci number.

Distance matrixes based on the Jaccard coefficient of genetic similarity (Jaccard 1908) were created, and UPGMA dendrograms were constructed based on the coefficients with free online software (http://genomes.urv.cat/UPGMA/) as well as heatmaps and PCA analyse, that were constructed in SRplot-Science & Research Plot (http://www.bioinformatics.com.cn/plot_basic_cluster_heatmap_plot_024_en).

RESULTS

BBAP population diversity. The degenerate primer pairs amplified 23 consistent bands, of which 21 were polymorphic (Figure 2). Fragment sizes ranged from 85 bp to 1 185 bp. A total of 218 bands were amplified in the set of 21 analysed accessions. Accessions of peanuts showed from six (accession 13) to sixteen (accession 18) bands. Four unique amplicons were identified: 626 bp for accession 4, 396 bp for accession 18, 123 bp for accession 18 and 112 bp for the accession 19 (Figure 2). Only the amplicons with lengths of 218 bp and 85 bp were amplified in all the analysed peanut accessions, providing a polymorphism of 91%.

Constructed dendrogram for BBAP polymorphism showed the separation of all peanut accessions except accessions 11 and 12. A total of three main branches were separated (Figure 3).

The most distinctive BBAP fingerprints were obtained for accessions 13 and 16, with a Jaccard coe-

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amplicon length/ accesion	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1185	-	-	-	-		-	-	-	-	-	-	-		-	-		-	-	-	-	-
1108													-			-					
978													-			-			-		
846													-			-			-	-	
626	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
613	-			-	-								-		-	-			-	-	
560	-				-								-			-			-		
535	-			-	-	-		-					-		-	-				-	
469	-	-	-	-	-	-	-	-	-	-	-	-		-		-	-	-	-	-	-
423		-		-			-		-				-		-						
396	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
348	-	-	-	-	-	-		-	-				-	-	-	-			-	-	-
218																					
204	-				-								-		-	-			-	-	-
196	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
174		-	-		-	-	-		-	-		-	-	-			-	-	-		-
162	-		-	-	-	-	-	-		-			-	-	-	-	-		-	-	-
139															-	-				-	
123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
112	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
103	-	-	-	-	-	-	-	_	-	-	-	-		-			-		-		-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	
85																					

Figure 2. Distribution of obtained BBAP (Bet v1 based amplicon polymorphism) fingerprints of *Arachis hypogaea* and two wild accessions by degenerated primer pairs. Unique fragments are marked green, fragments amplified in at least two accessions are marked grey and "–" stands for the missing band

fficient of genetic similarity of 0.36, then accession 19, with an average Jaccard coefficient of genetic similarity to all other analysed accessions of 0.37 and accessions 15 and 20, with a Jaccard coefficient of genetic similarity of 0.7. Calculated Jaccard coefficients of genetic similarity for analysed accessions of peanuts ranged from 0.20 (accessions 10 and 13) up to 1 (accessions 12 and 11).

A circular cluster heatmap of amplified band distribution among the peanut accessions is illustrated in Figure 4. The most abundant amplicons generated in all of the analysed accessions of peanuts were in the range of length of 80–220 bp.

PBAP population diversity. The profilin-based primer pairs amplified 30 consistent bands, of which 29 were polymorphic (Figure 5). Fragment sizes

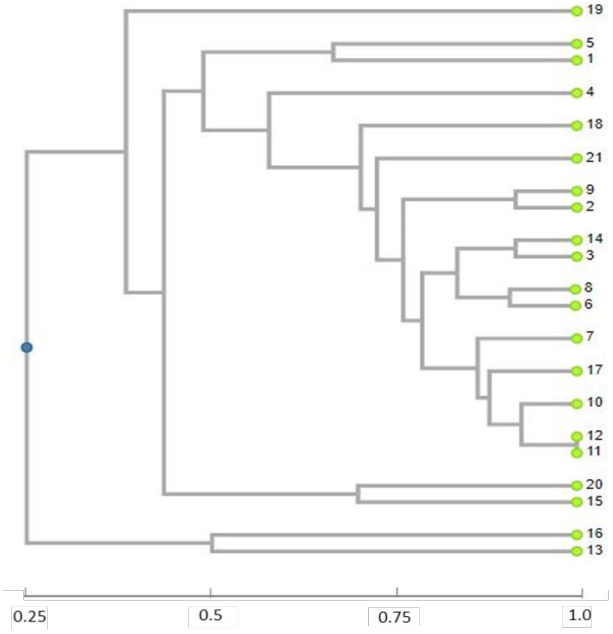

Figure 3. UPGMA dendrogram of Jaccard genetic similarity values among 19 *Arachis hypogaea* and two wild accessions for BBAP (Bet v1 based amplicon polymorphism) fingerprints

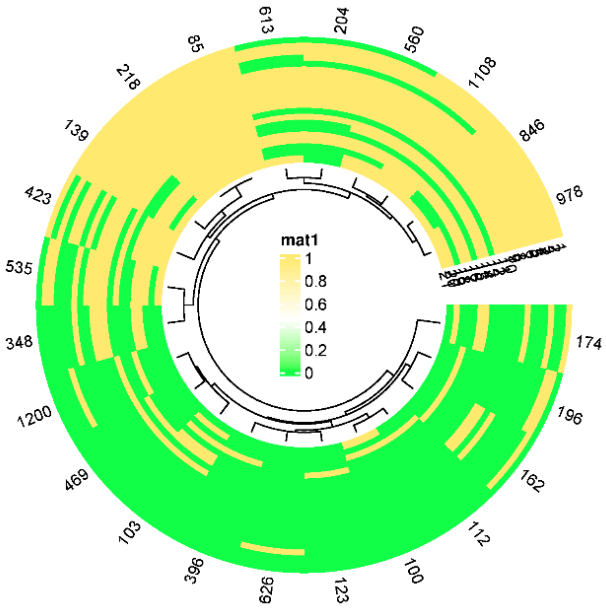

Figure 4. Circular cluster heatmap of amplicon length distribution among 19 *Arachis hypogaea* and two wild accessions for BBAP (Bet v1 based amplicon polymorphism)-generated fingerprints. Yellow colour stands for the presence of an amplicon of appropriate length

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amplicon length/ accesion	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1642		-			-							-	-		-	-			-		
1275													-			-		-			
1203												-	-			-			-		-
879					-	-	-	-	-	-	-	-	-	-	-		-	-			
804													-			-					
766		-	-	-	-	-	-	-	-	-	-	-	-	.	-	-	-	-	-		-
683					-	-	-					-	_		-	-			-		-
612			-			-	-	-	-	-	-		-		-	-			-	-	-
521	-	-	-		-	-					-		-		-	-		-	-	-	-
482					-	-							-			-		-			
460	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-		-	-	-
434																-					
365	-	-	-	-	-	-	-		-		-	-	-	-		-		-		-	-
322	-	-	-	-	-				-	-	-		-			-		-	-	-	-
299	-	-	-	-			-		-	-	-		-		-	-	-	-			-
286	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
258					-	-		-					-		-	-	-	-	-		-
220												-	-			-				-	
214	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
196		-	-		-	-		-			-					-			-	-	
190	-	-	-									-	-	-	-		-	-	-	-	-
173	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
163	-		-	-		-	-		-	-	-	-		-	-	-	-	-	-	-	-
143	-	-	-	-	-	-		-	-	-	-	-	-	-		-	-	-		-	-
134	-		-			-	-		-	-	-	-	-	-	-			-			-
118		-	-	-	-	-	-	-	-	-	-	-		-	-		-	-		-	-
100																					
96	-	-	-	-			-		-	-	-	-	-	-	-		-	-	-	-	-
90	-	-		-		-	-	-	-	-	-	-			-		-				-
80		-	-	-			-	-	-	-	-	-		-	-		-		-	-	

Figure 5. Distribution of obtained PBAP (profilin based amplicon polymorphism) fingerprints of *Arachis hypogaea* and two wild accessions by profilin-based primer pairs. The unique fragment of accession 20 is marked green, fragments amplified in at least two accessions are marked grey and "–" stands for the missing band

ranged from 80 bp to 1 642 bp. A total of 271 bands were amplified in the set of 21 analysed accessions. Accessions showed from seven (accession 13) up to seventeen (accession 8). Only one unique amplicon of size 286 bp for the accession 20 was identified. The most distinctive fingerprints were present in accessions 13 and 16. Only an amplicon with a length of 100 bp was amplified in all of the analysed accessions, providing a polymorphism rate of 97%. The constructed dendrogram for PBAP polymorphism distinguished all the peanut accessions studied (Figure 6).

A total of seven main branches were separated. The most distinctive PBAP fingerprints were obtained again for accessions 13 and 16 with the Jaccard coefficient of genetic similarity of 0.33. All the other analysed accessions were in a quite narrow range of genetic similarity. Calculated Jaccard coefficients of genetic similarity for analysed accessions of peanuts ranged from 0.09 (accessions 16 and 17) to 0.93 (accessions 9 and 10).

A circular cluster heatmap of amplified allele distribution among the analysed peanut accessions for the PBAP technique exhibited a more polymorphic pattern than the BBAP, considering the length of amplified bands (Figure 7). The significant proportion of generated amplicons was between 100, 220, 400–500, 804 and 1 200–1 300 bp.

VBAP population diversity. The degenerate primer pairs amplified 34 consistent and polymorphic bands (Figure 8). Fragment sizes ranged from 83 bp to 1 257 bp. A total of 221 bands were amplified in the set of 21 analysed accessions.

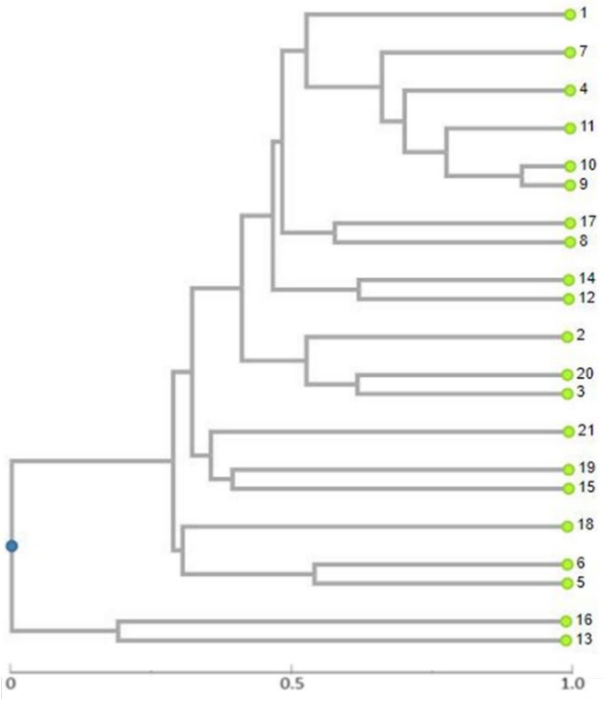

Figure 6. UPGMA dendrogram of Jaccard genetic similarity values among 19 *Arachis hypogaea* and two wild accessions for PBAP (profilin based amplicon polymorphism) fingerprints with average values of Jaccard coefficients of genetic similarity among the clusters

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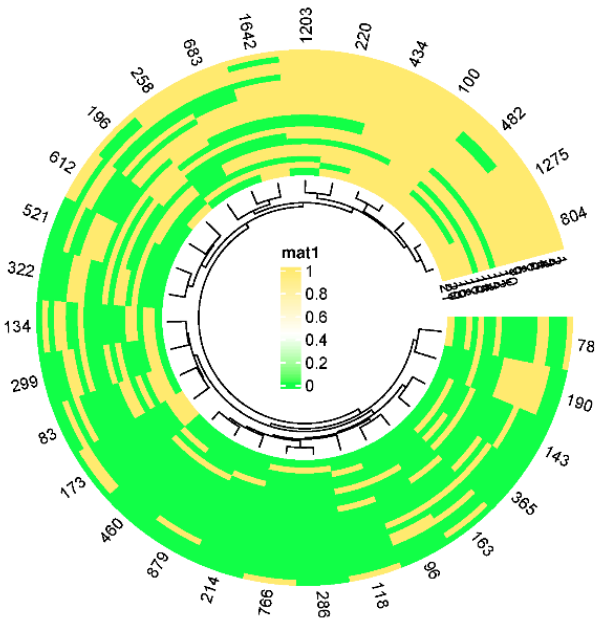

Figure 7. Circular cluster heatmap of amplicon length distribution among 19 *Arachis hypogaea* and two wild accessions for PBAP (profilin based amplicon polymorphism)-generated fingerprints. Yellow colour stands for the presence of an amplicon of appropriate length

Peanut accessions showed from four (accession 13) to fourteen (accessions 4, 9 and 21) bands. Seven unique amplicons were identified: 822 bp for accession 18, 697 bp for accession 3, 504 bp for accession 4, 475 bp for accession 1, 172 bp for accession 6, 141 bp for accession 14 and 89 bp for accession 21. None of the loci was amplified in all the analysed accessions, providing a polymorphism level of 100%.

Similarly to PBAP, the dendrogram from VBAP (Figure 9) was able to distinguish all analysed accessions. A total of five main branches were separated.

The most distinctive VBAP fingerprints were obtained again for accessions 13 and 16 with the Jaccard coefficient of genetic similarity 0.25. These two accessions produced distinctive fingerprints for all the marker techniques based on the allergen-coding genes used in this study. However, previously, when analysed by iPBS markers, they were similar to the other accessions (Montero-Torres et al. 2020). The other 19 accessions were joined together at the Jaccard similar coefficient 0.32 value. Calculated Jaccard coefficients of genetic similarity for analysed accessions of peanuts ranged from 0.06 (accessions 13 and 14; accessions 2 and 16) to 0.92 (accessions 9 and 12).

The circular cluster heatmap of the analysed accessions for the amplified bands through the VBAP

amplicon length/ accesion	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1257		-	-										-		-	-			-		
918													-			-					
822	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
746	-	-	-										-		-						
697	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
676			-										-			-					
607	-	-	-	-	-	-	-	-	-	-	-	-		-	-		-	-	-	-	-
563	-	-		-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
536			-										-		-	-					
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346		-	-	-	-	-	-		-	-		-	-		-	-		-	-	-	-
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153	-	-	-	-	-	-	-	-	-	-	-	-		-		-	-	-	-	-	-
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89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
83													-					-		-	

Figure 8. Distribution of obtained VBAP (vicilin based amplicon polymorphism) fingerprints of 19 *Arachis hypogaea* L. and two wild accessions by vicilin-based primer pairs. Unique fragments are marked green, fragments amplified in at least two accessions are marked grey and "–" stands for the missing band

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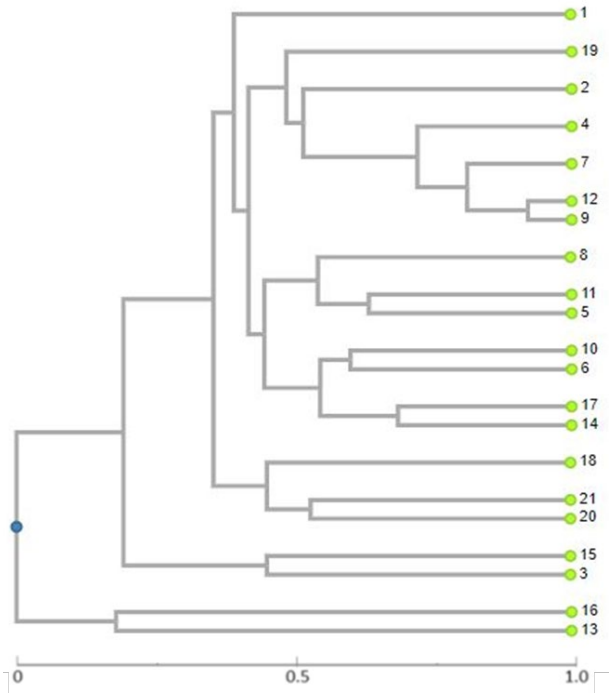

Figure 9. UPGMA dendrogram of Jaccard genetic similarity values among 19 *Arachis hypogaea* and two wild accessions for VBAP (vicilin based amplicon polymorphism) fingerprints

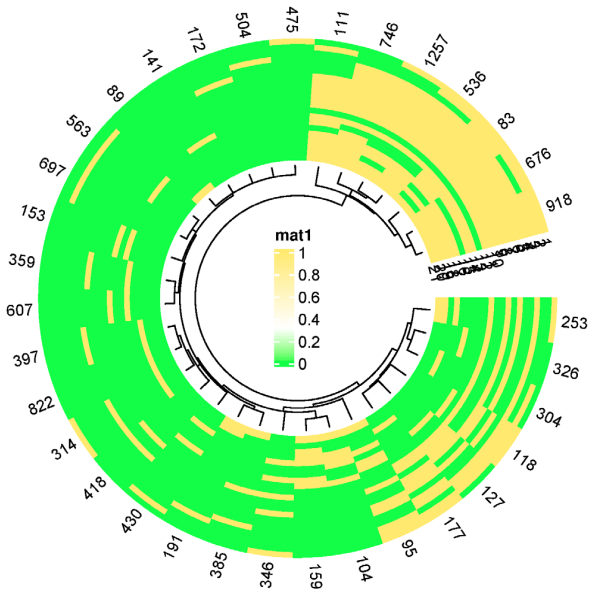

Figure 10. Circular cluster heatmap of amplicon length distribution among 19 *Arachis hypogaea* and two wild accessions for VBAP (vicilin based amplicon polymorphism) generated fingerprints. Yellow colour stands for the presence of an amplicon of appropriate length

technique showed that the generated amplicons were distributed evenly. Additionally, it indicated that among all of the techniques used in this study, VBAP was the most polymorphic for amplified loci in the analysed accessions (Figure 10).

Comparing the techniques used in the study, VBAP was the most effective in distinguishing the analysed peanut accessions (Table 2). For BBAP, two of the analysed accessions provided the same fingerprinting pattern, and this technique was not able to distinguish them. In the case of PBAP, 58% of the analysed peanut accessions had Jaccard similarity indices higher than 0.5, and the discrimination power of this technique was lower when compared to the other techniques used here.

The ability of the used markers to detect polymorphisms was comparable, with an average PIC value of 0.47. VBAP markers provided the best effectiveness for the polymorphism analysis of *Arachis* variability, as the marker index value was higher among the three used techniques. The highest discrimination power index was also obtained for VBAP, corresponding to the most distinct fingerprints for individual accessions. In all the marker techniques used in the study, peanut accessions Blanco quinsancho and Oclliri Colorado were separated from all others.

PCA analysis was performed to compare the generated polymorphic profiles of individual techniques used in the study to see the differences in the amplified fingerprints (Figure 11). A clear separation of all of them

Table 2. Characteristics of fingerprints obtained by BBAP (Bet v1 based amplicon polymorphism), PBAP (profilin based amplicon polymorphism) and VBAP (vicilin based amplicon polymorphism) markers

Marker	Polymorphism (%)	PIC	AN	MI	DI	D
BBAP	91	0.5	10.38	9.58	0.49	0.79
PBAP	97	0.49	12.9	13.73	0.58	0.3
VBAP	100	0.43	10.52	14.62	0.42	0.9

PIC – polymorphism information content; AN – average number of amplified alleles per accession; MI – marker index; DI – diversity index; D – discrimination power

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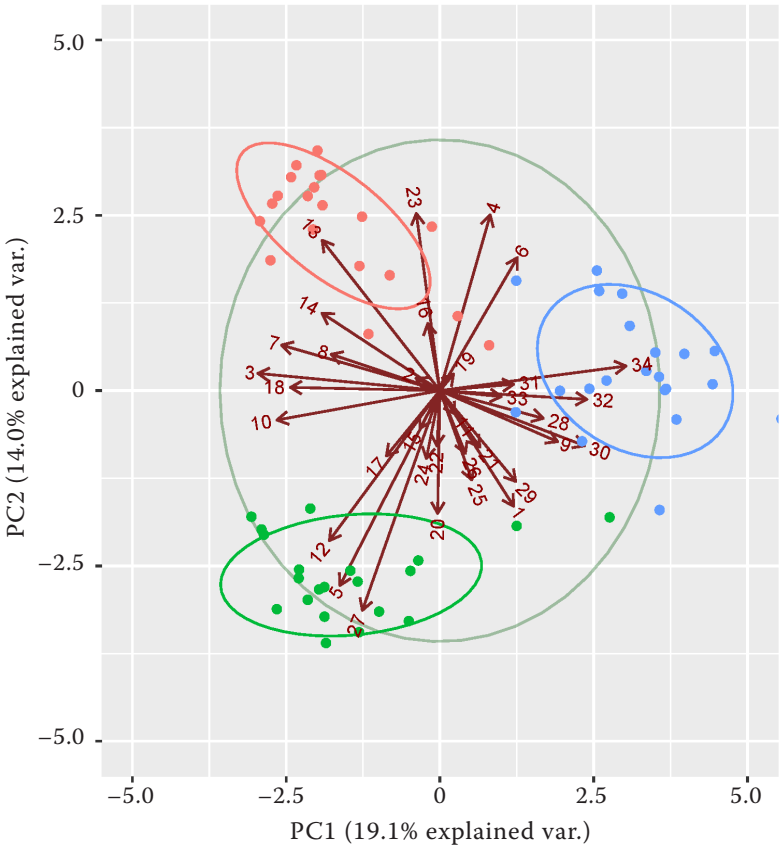

Figure 11. Principal component analysis (PCA) analysis of obtained polymorphism for BBAP (Bet v1 based amplicon polymorphism), PBAP (profilin based amplicon polymorphism) and VBAP (vicilin based amplicon polymorphism) fingerprints

and the visualisation of intra-accession polymorphism of individual peanut accessions was obtained.

ANOVA with post-hoc Tukey *HSD* (honestly significant difference) test was performed to compare the marker techniques used in this study (Table 3). PBAP and VBAP-generated polymorphic patterns were not significantly different. BBAP technique fingerprints were significantly different at $P < 0.01$ when compared to both other techniques used.

DISCUSSION

In silico comparison of the amino-acid sequences reported for Bet v 1 allergen shows a very variable identity among plant species (Breiteneder and Ebner 2000). This provides an option to use these regions as DNA-based markers for analysing the variability of these genes in plants. The homology of amino acid sequences in the region of forward primer for BBAP strategy is relatively high and includes the confirmed epitope for IgE (Uehara et al. 2001). Reverse primers amplify a variable region of the year-10 gene of Bet v 1 and match the amino acid variability at position 119 of Bet v 1 protein (P15494) (Breiteneder and Ebner 2000). In this study, different peanut accessions were analysed to prove the efficiency of the *in silico*-generated markers to provide polymorphic fingerprints among them.

Table 3. Results of Tukey *HSD* (honestly significant difference) for individual marker techniques used in this study to reveal polymorphism in the 21 accessions

Treatments pair	Q statistic	*P*-value	Inference
BBAP *vs.* PBAP	5.045	0.0011268	$P < 0.01$
BBAP *vs.* VBAP	7.29	0.0010053	$P < 0.01$
PBAP *vs.* VBAP	2.74	0.2544528	not significant

BBAP (Bet v1 based amplicon polymorphism); PBAP (profilin based amplicon polymorphism); VBAP (vicilin based amplicon polymorphism)

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Ara h 8 is the Bet v 1 homologous allergen of peanuts (Mittag et al. 2004). As the Bet v 1 is a model allergen, it is well-characterised along with many of its homologs across the plant species. Ara h 8 is a minor food allergen that is responsible for oral allergy syndrome (OAS). Other airborne allergens cause sensitisation, leading to an allergic reaction (Type 2) to ingested foods for Ara h 8 (Hurlburt et al. 2013). The structure of Bet v 1 allergen has been established with an unusual characteristic, as it does not have a globular, hydrophobic core (Gajhede et al. 1996) but a curved, seven-stranded β-sheet that wraps around a long C-terminal α-helix, further separated by two helices. Actually, many of the structures of proteins from food that cause OAS have been determined (Alessandri et al. 2020). Interestingly, in most cases, the structures are greatly similar to Bet v 1. However, they differ in their primary amino acid sequences (Berkner et al. 2009), which is the base of the BBAP polymorphism-generating background. The proteins Bet v 1-like superfamily includes a class of proteins called PR-10 for pathogenesis-related class 10. Among these proteins, Ara h 8 has the lowest sequence identity to Bet v 1 (48%). Previously, degenerate primers that anneal a variable and conserved part of PR-10 protein homologues genes in the BBAP technique were applied to analyse intraspecific variability of *Malus domestica* Borkh. cultivars (Speváková et al. 2021). Amplicons were generated and formed relatively monomorphic profiles, indicating the stability of the given isoforms of Bet v 1 within the selected apple cultivars. Bet v 1 homologs in the apple genome possess a high protein structure homology due to short amino acid sequences, which are highly conserved among plant species, resulting in a very similar or identical protein structure (Seutter von Loetzen et al. 2012), despite the relatively variable nucleotide sequences of the ypr10 genes (genomic similarity 50 –> 90%) (Fernandes et al. 2013). Here, BBAP fingerprints generated were polymorphic.

The Ara h 5 (peanut allergen) is a member of the profilin family. Profilins are actin-binding molecules that are reported to be a well-known plant panallergen group (Valenta et al. 1991). Profilin was first documented as an allergen found in birch pollen, called Bet v 2 (Valenta et al. 1991), and is recognised for causing an allergic reaction to pear, peach, apple, melon, tomato, celery, pumpkin seeds, and peanuts (Breiteneder and Ebner 2001). Completely different amplicon profiles of PBAP were obtained for some of the previously analysed legume species (Klongová et al. 2021), where the amplicons generated were within a range of 46 bp to 669 bp, and this technique differentiated species analysed in the study. Here, PBAP fingerprints of peanuts were distributed among 30 different loci, with their length ranging from 78 bp to 1 642 bp.

Ara h 1 is a cupin-type allergen, homologous to a vicilin-based group of allergens present in the peanut genome. This group of allergenic seed storage proteins classified as 7S globulins are found in a wide range of plants but are often termed as *vicilins* due to the dominating presence of the Viciae group in the legumes (Astwood et al. 2002). Actually, 28 isoallergens are reported in the databases for which different IgE-binding epitopes were reported previously. Based on its sequential similarity, Ara h 1 is cross-reactive with allergens of lentils and peas, walnut, cashew, and hazelnut (Barre et al. 2008), lupine (Dooper et al. 2009), and intraspecifically with other Ara h allergens (Croote et al. 2018).

In the case of VBAP, its transferability for legume species was reported previously, as different amplicon profiles were obtained for the legume species (Klongová et al. 2021), ranging from 83 bp to 253 bp, and the VBAP marker was able to differentiate the species studied effectively. Similarly to the current study, the shortest and the longest amplified fragments were of the same lengths, 83 bp 1 257 bp, respectively. Ara h 1 and Ara h 3 belong to the cupin superfamily, a functionally highly diverse protein containing at least 61 member families (Dunwell et al. 2004). Because of the abundance of vicilin homologs in the genomes of legumes, VBAP was designed, and this marker technique should provide an effective tool for genomic variability analysis in legumes.

The results obtained from the current study will provide valuable data for the analysis of the *A. hypogaea* germplasm variability concerned specifically with coding regions of allergens. Additionally, it will form a basis for future molecular and breeding studies aiming to explore allergen variability among peanut accessions.

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