Acta fytotechn zootechn, 24, 2021(Monothematic Issue: Actual Problems in Agrobiology): 1–6
http://www.acta.fapz.uniag.sk

Original Paper

Variability of DNA based amplicon profiles generated by Bet v 1 homologous among different vegetable species

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Article Details: Received: 2020-10-07 | Accepted: 2021-02-08 | Available online: 2021-02-28

https://doi.org/10.15414/afz.2021.24.mi-apa.1-6

Pathogen-related 10 is a group of proteins derived from various plant species that contains homologues of Bet v 1, major birch-pollen allergen, which are able to cross-react with an IgEs of sensitive patients and invoke allergic reactions. Despite new sequencing technologies and the amount of data generated daily, PR-10 proteins have not been discovered in every plant species. To describe genomic variability by the -omics sciences, it is necessary to screen an undiscovered members of the group, map ypr-10 genes in genome and determine sequence variabilities. In this study Bet v 1 based amplified profile (BBAP) method was used to analyse the variability of 14 vegetable species (*Brassica oleracea* in 4 varieties) and to compare generated polymorphism among them. For all of the analysed species, a total number of generated amplicons was 162. The most different profiles were generated by *Pastinaca sativa* and *Capsicum annuum*, the highest similarity was between *Apium graveolens* and *B. oleracea* var. *capitata*. New type of genic molecular markers was applied successfully and proved to be an effective technique to map variability of PR-10 group.

Keywords: PR-10, Bet v 1 homologue, vegetable, DNA marker, cross-reaction, allergy, genic molecular marker

1 Introduction

Association between edible fruits and vegetables and hypersensitivity to pollen allergens have been described already in the middle of the last century (Caballero and Martín-Esteban, 1998). Cross-reactions between major birch-pollen Bet v 1 allergen could be included in the most often where homologues among different species in fruits and vegetables as apple (Mal d 1), cherry (Pru av 1), celery (Api g 1), carrot (Dau c 1), etc. belong. These potentially allergic proteins are classified as pathogen-related proteins, specified in a group PR-10, also known as Bet v 1 – homologue proteins (Helbling et al., 1993; Breiteneder and Ebner, 2000). Up to date, an exact function of PR-10 proteins is very poorly understood, but an expression is initiated in the presence of biotic (pathogens) (Pühringer et al., 2000; Robert et al., 2001; McGee et al., 2001) or abiotic (cold, salinity, drought) stress (Moons et al., 1997; Wisniewski et al., 2004; Park et al., 2004), therefore the protective role of the proteins is predicted. On the other hand, many studies are focused on its ability to evoke an allergic reaction in hypersensitive patients allergic to birch pollen (Elisyutina et al., 2019; Kopac et al., 2011) or structure of homologue proteins (Fernandes et al., 2013).

Interestingly, genomic similarity of PR-10 protein homologues varies from 50 to >90%, what not correspond to the ability to evoke cross-reaction with Bet v 1 allergen. The key of cross-reactions are epitopes, short amino-acid highly conserved sequences where the specific IgE is bound. (Fernandes et al., 2013). Amino-acid and nucleotide sequence data of different PR-10 proteins became highly important for -omics based studies, however the known sequences are very limited, especially the nucleotide ones. Clinically most relevant cross-reactive species as apple, peach or celery are studied better than less present, but the clinical relevancy may be slightly distorted, because it is often the case that patients do not report allergy suspicion and only avoid the problematic food ingredient. Cross-reactivity among pollen and food allergens is studied mainly for setting the base of food safety with the aim to understand how sensitization to specific allergen starts cross-reactivity to homologue allergens in other foods (McClain, 2017). Lorenz et al. (2009)

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reported, that the concept of homologue groups is dynamic and a new members and knowledge about them should be added. Based on the statement, Heath and Hutchings (2015) studied cross-reactivity and structural homology between Bet v 1 allergen and several potential homologue proteins, not included into PR-10 group. They found Fag s 1 (beech) sequence identity and ability to cross reactivity with Bet v 1 is higher than Que a 1 (oak), which is already a member of PR-10.

Most of the allergen homology studies are oriented to proteins and the knowledge of their genomic variability is still limited. PR-10 genes that codes Bet v 1 – homologue proteins have different level of genomic homology (Žiarovská and Zeleňáková, 2016), but conserved sites of these genes have the potential to be used as DNA based markers that can reveal the sequential variability among different plant species (Žiarovská and Zeleňáková, 2019). This study is focused on analysing the Bet v 1 based amplified profile (BBAP) variability among vegetable species often consumed and easily accessible on the market in Central Europe.

2 Material and methods

2.1 Biological material and DNA extraction

18 different types of vegetables (Table 1) were processed into samples and frozen until DNA extraction. Total genomic DNA was extracted from 100 mg of frozen and grinded plant tissue by GeneJET Plant Genomic DNA Purification Kit (ThermoFisher®). Quality and quantity of sample's genomic DNA was checked by Nanophotometer P360 (Implen) and samples were diluted to 1–5 ng μl^{-1} (depends on the type of tissue and its contaminants). Because of different DNA concentrations, DNAs of individual species were proved for its quality in PCR when using of universal ITS primers (White et al., 1990).

2.2 PCR analysis and dendrogram construction

Primers that match the coding region of ypr-10 gene (NCBI: AJ289771.1) were designed according Žiarovská and Zeleňáková (2016) with the degenerated reverse primer representative four individual primers. PCR products were amplified by MasterMix Robust HS Elizyme (Elizabeth Pharmacon) with 2–10 ng DNA and 400 nM of each primer. Thermal profile for PCR was as follows: initiating denaturation at 95 °C for 5 min; followed by 40 cycles of denaturation

Table 1 Scientific/common vegetable names of species used in BBAP analysis

Family	Species		Vegetable
Alliaceae	*Allium cepa*		onion
Amaranthaceae	*Beta vulgaris*		beetroot
	Spinacia oleracea		spinach
Apiaceae	*Apium graveolens*		celery
	Daucus carota		carrot
	Pastinaca sativa		parsnip
	Petroselinum crispum		parsley
Asteraceae	*Lactuca sativa*		lettuce
Brassicaceae	*Brassica oleracea*	var. *capitata*	cabbage
		var. *botrytis*	cauliflower
		var. *gongylodes*	kohlrabi/cabbage turnip
		var. *italica*	broccoli
Cucurbitaceae	*Cucurbita maxima*		pumpkin (Hokkaido)
	Cucurbita pepo		zucchini
Convolvulaceae	*Ipomoea batatas*		sweet potato
Lauraceae	*Persea americana*		avocado
Solanaceae	*Solanum tuberosum*		potato
	Capsicum annuum		pepper bell

at 95 °C for 45 s, annealing primers at 54 °C for 45 s and elongation at 72 °C for 35 s; ended by final elongation at 72 °C for 10 min.

Generated amplicons were loaded on 1.5% agars gels, separated by electrophoresis and stained by GelRed™ (Biotium). Profiles of BBAP were analysed by free available software GelAnalyzer (www.gelanalyzer.com) and binary matrices of amplicon presence were prepared. Nei-Li coefficient of genetic distance was used to calculate basic relationships among individual species and a dendrogram was constructed by UPGMA method.

3 Results and discussion

PR-10 is a variable group of proteins originated from different plant taxa, that members are able to enter into cross-reactions thanks their homology in conserved epitopes (Andersen et al., 2009). In silico comparison of member's amino-acid sequences shows variable identity among plant species (Breiteneder and Ebner, 2000) what suggests an option to use the region as a molecular marker for natural variability of ypr-10 genes in plants. In this study were analysed 18 different types of vegetables (14 species plus 1 species in 4 varieties) often consumed and easily accessible on the market in Central Europe. Forward primer for BBAP was designed to match region located between β3 and β4 of expressed protein. Homology of amino acid sequences in this region is relatively high and includes epitope for IgE (Uehara et al., 2001). Reverse primer was designed to match relatively variable region of ypr-10 gene when compared to Bet v 1 with the amino acid variability at the position 119 of Bet v 1 protein (P15494) (Breiteneder and Ebner, 2000).

For all of the analysed species of vegetable the amplification of BBAP fingerprints was successful and several PCR products created length polymorphism of Bet v 1 homology genes. Total number of amplicons was 162, average and median were identical: 29,5. Polymorphisms were very variable in count of amplicons, only 15 amplicons were amplified in the case of *Allium cepa*, the most numerous were *Ipomoea batatas* with 41 amplicons. The most often length was 720 bp, the most unique were amplicons of 1,450 bp and 680 bp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.000																	
2	0.756	0.000																
3	0.872	0.857	0.000															
4	0.744	0.795	0.811	0.000														
5	0.763	0.824	0.719	0.833	0.000													
6	0.694	0.813	0.833	0.794	0.793	0.000												
7	0.760	0.910	0.746	0.775	0.803	0.614	0.000											
8	0.815	0.825	0.849	0.770	0.804	0.872	0.680	0.000										
9	0.818	0.855	0.754	0.863	0.841	0.898	0.839	0.808	0.000									
10	0.765	0.844	0.836	0.728	0.775	0.851	0.743	0.733	0.806	0.000								
11	0.937	0.927	0.961	0.864	0.918	0.911	0.875	0.684	0.760	0.759	0.000							
12	0.822	0.878	0.897	0.721	0.868	0.944	0.893	0.815	0.714	0.741	0.746	0.000						
13	0.708	0.802	0.844	0.647	0.920	0.690	0.811	0.875	0.816	0.762	0.935	0.640	0.000					
14	0.842	0.794	0.906	0.750	0.871	0.897	0.803	0.961	0.968	0.746	0.796	0.684	0.733	0.000				
15	0.875	0.806	0.882	0.763	0.848	0.903	0.938	0.927	0.791	0.707	0.849	0.625	0.797	0.818	0.000			
16	0.681	0.860	0.780	0.689	0.800	0.711	0.747	0.884	0.877	0.820	1.000	0.745	0.656	0.750	0.714	0.000		
17	0.882	0.833	0.893	0.813	0.963	0.920	0.925	0.953	0.927	0.810	0.951	0.882	0.851	0.889	0.862	0.750	0.000	
18	0.825	0.889	0.912	0.842	0.909	0.839	0.877	0.782	0.731	0.787	0.774	0.700	0.772	0.909	0.829	0.810	0.897	0.000

Figure 1 Genetic distances of species analysed by BBAP in the study. Numbers of species are as follows: *Petroselinum crispum*, *Capsicum annuum*, *Daucus carota*, *Solanum tuberosum*, *Spinacia oleracea*, *Apium graveolens*, *B. oleracea* var. *capitata*, *Lactuca sativa*, *B. oleracea* var. *italica*, *B. oleracea* var. *botrytis*, *Allium cepa*, *Cucurbita pepo*, *Cucurbita maxima*, *B. oleracea* var. *gongylodes*, *Ipomoea batatas*, *Pastinaca sativa*, *Beta vulgaris*.

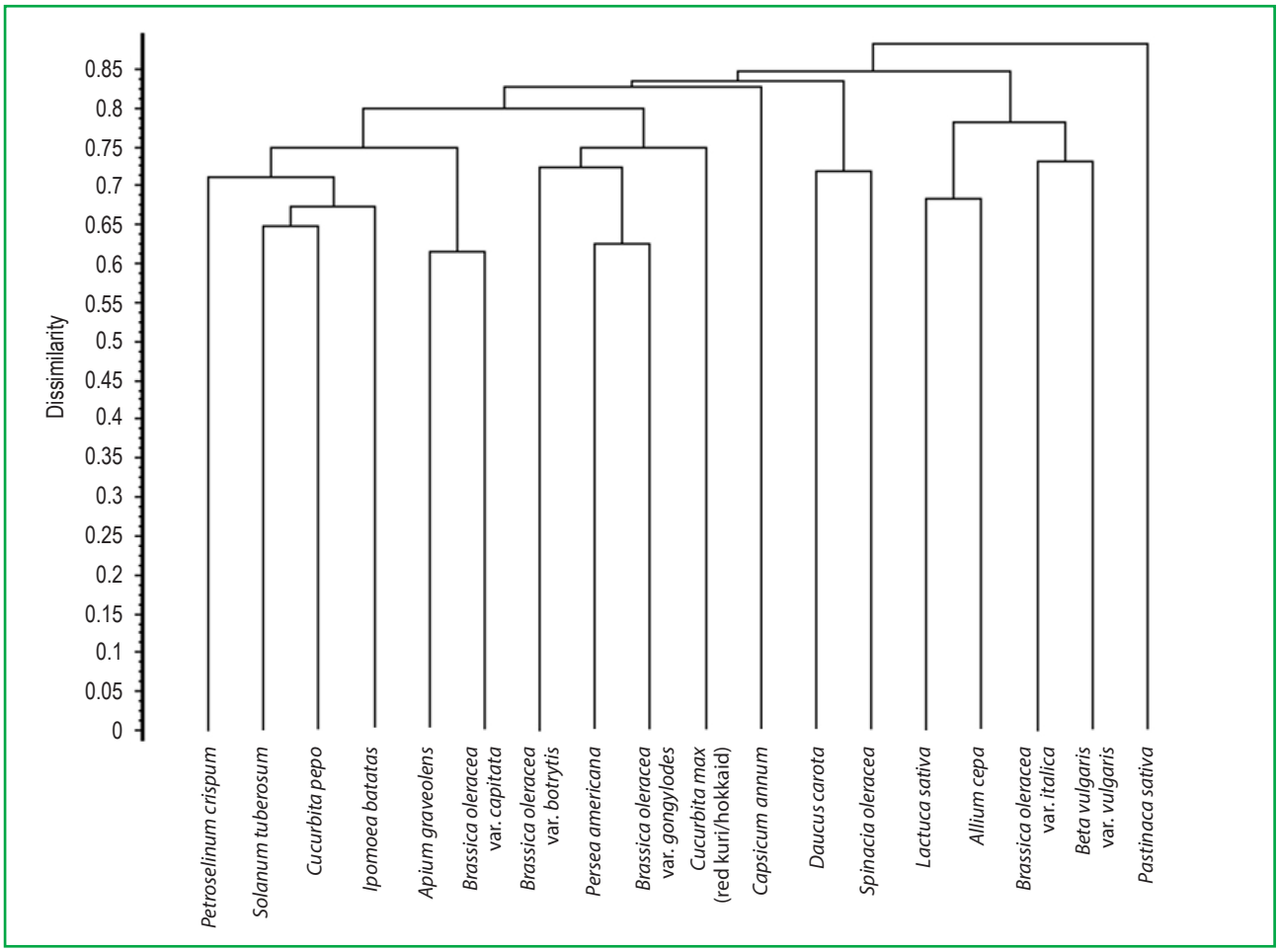

Figure 2 Dissimilarity dendrogram for length polymorphism of Bet v 1 homology genes in analysed species of vegetable

Pastinaca sativa amplified 21 amplicons and the profile quite differs from any of the other species. Genetic distances of *P. sativa* and other analysed species varied from 0.750 to 0.963, the average was 0.882. On the other hand, the most similar shows to be *A. graveolens* and *B. oleracea* var. *capitata* with following identical lengths: 70 bp, 100 bp, 200 bp, 220 bp, 290 bp, 560 bp and 650 bp; genetic distance between the species was only 0.613.

B. oleracea amplified 4 different BBAP profiles for each variety, var. *italica* and var. *gongylodes* amplified 30 amplicons each, var. *botrytis* 35 and var. *capitata* 28. Varieties *italica*, *botrytis* and gongolydes were similar in length 1100 bp, genetic distances of *B. oleracea* 4 varieties by Nei-Li coefficient varied from 0.743 between var. *botrytis* and var. *capitata* to 0.968 between var. *gongylodes* and var. *italica*.

Genetic distance analysis of Nei-Li coefficient results show a narrow range among all of the analysed species where the difference was only 0.36, but the genetic was relatively high (Figure 1) what correspond to the findings of variability among plant PR-10 proteins (Freitas et al., 2003; Uehara et al., 2001). The most different amplicon profiles with the absolute Nei-Li distance was calculated for *Allium cepa* and *Ipomoea batatas*.

Separated PCR products were transformed into binary matrices used to create dissimilarity dendrogram with cophenetic coefficient 0.78 (Figure 2). Analysed species were clustered into 5 subclusters with the alone standing species dissimilar to the others – *Capsicum annuum* and *Pastinaca sativa*. No specific grouping according the phylogenetic relationships was typical in the clusters of dendrogram, what assumed BBAP as an effective technique for discrimination of the plant species.

Reports and records of patients suffering from food-pollen allergies speak of a different ability to induce a specific cross-reaction with an allergen. As mentioned above, PR-10 is a group of proteins involving variability not only at the species level (Fernandes et al., 2013), the variability is enriched by isoforms (Gao et al., 2005). As Fernandes et al. (2013) mentioned, sequence identity of "classic" PR-10 proteins is 50% and more, indicating the high variability of the group.

The ever-increasing knowledge of gene sequences brought new possibilities in breeding and crop improvement by genic molecular markers using the coding space (Varshney, 2010). Genic molecular markers appear to be more suitable for marker-assisted selection or comparative mapping, etc. (Varshney et al., 2007) used for transcript map construction in chickpea (Gujaria et al., 2011) or for the diversification of the genotypes of millet and its calcium transporters (Nirgude et al., 2014).

4 Conclusion

A new type of DNA based markers of coding region was firstly applied here for vegetable species and an effectivity of these technique was proved as universal in its applicability.

Acknowledgments

This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

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