STUDIES CONCERNING THE ALLERGENICITY OF DIFFERENT APPLE VARIETIES CULTIVATED IN REPUBLIC OF MOLDOVA

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Abstract

The allergenic properties of apple varieties are very different and mechanisms which ensure these difference are not fully elucidated. In this study was estimated transcriptional activity of four allergen genes (Mal d 1.01, Mal d 1.02, Mal d 1.03 and Mal d 1.04) in peel and pulp of seven apple varieties, widely presented in the market in Republic of Moldova. The maximum values of transcript accumulation were attested for Mal d 1.01 cluster. Mal d 1.03 and Mal d 1.02 had intermediate expression levels. Mal d 1.04 clusters had minimal transcriptional activity values, which in most cases were very low or undetectable. In base of obtained results, have been revealed the highly allergenic (Golden delicious, Florina and Mantuaner) and hypoallergenic (Richard (Gloster)) varieties.

Key words: apple, allergenicity, gene expression.

Apple (*Malus domestica* L. Borkh) is one of the most consumed fruit in the word. Due to antioxidant capacity and important micro-nutrients content, the apple is included in healthy diet and could contribute to reduction of the risks of lung cancer and cardiovascular diseases (Knekt P. *et al*, 1996, 2000; Le Marchand L. *et al*, 2000). However, apple fruit are reported to be frequently responsible for allergic reactions with wide variety of symptoms: from mild and localized reactions like an oral allergic syndrome (OAS) to severe such as an anaphylactic shock (Hoffmann-Sommergruber K., 2005). Two models of apple allergy were described in Europe. Thus, in the Northern and Central Europe apple allergy occurs mainly due to IgE cross-reactivity to the Bet v 1 major birch pollen allergen, which has a high degree of homology with apple allergen Mal d 1 (Son D.Y. *et al*, 1999). This type of food allergy is found frequently in patients sensitive to birch pollen and causes mild local symptoms. Unlike first type of allergy, in the Mediterranean region apple allergy is often diagnosed in patients who are not sensitive to the pollen (Fernandez-Rivas M. *et al*, 2006). This form of allergy causes more severe symptoms and it is determined mainly by allergen Mal d 3.

In most cases fruit allergies are due to presence of different classes of Pathogenesis-Related proteins, such as class 10 (PR-10), class 14 Lipid Transfer Proteins (LTP, PR-14), class 5 Thaumatine-like proteins (TLP, PR-5), classes 3 and 4 chitinases (PR-3 and PR-4), class 2 β-1,3-glucanases (PR-2) or profilins (Hoffmann-Sommergruber K., 2002).

Four main classes of allergenic proteins are described in apple:

- Mal d 1 (PR-10), the major apple allergen, homologue of Bet v 1 protein from birch pollen with molecular weight 17.5 kD (Botton A. *et al*, 2008, 2009);
- Mal d 2 (PR-5), thaumatine-like protein with molecular weight 23 kD (Gao Z.S. *et al*, 2005a);
- Mal d 3 (PR-14), Lipid Transfer Protein with molecular weight 9 kD (Sancho A.I. *et al*, 2006b);
- Mal d 4 (profilin), homologue of Bet v 2 protein from birch pollen with molecular weight 14 kD (Gao Z.S. *et al*, 2005a).

Each family of proteins is encoded by several genes or gene clusters.

Genetic mapping studies revealed 31 genes encoding Mal d 1 protein isoforms (Pagliarani G. *et al*, 2012), which are classified into four groups: Mal d 1.01, 1.02, 1.03 and 1.04 (Gao Z.S. *et al*, 2005b; Puehringer H.M. *et al*, 2003). The expression level of these genes assessed by qRT-PCR revealed that 11 Mal d 1 genes were not expressed in fruits, thus, demonstrating the tissue-dependent expression. These data were obtained for ten varieties of apples (Florina, Gala, Santana, Elstar, Elise, Golden Delicious, first, Jonathan Cox

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and Ingrid Marie) with different degree of allergenicity (Pagliarani G. *et al*, 2013).

Also, the accumulation of both proteins and gene transcripts of *Mal d 1* in fruits has been associated with various factors such as the genotype, the ripening stage, cultivation and storage conditions (Hsieh L.S. *et al*, 1995; Botton A. *et al*, 2009; Matthes A. *et al*, 2009; Sancho A.I. *et al*, 2006a; Sancho A.I. *et al*, 2006b; Zuidmeer L. *et al*, 2006).

Investigations regarding allelic diversity of *Mal d 1* genes revealed highly conservative (*Mal d 1.01* and *Mal d 1.02*) and variable (*Mal d 1.04*, *Mal d 1.05* and *1.06 A, B* and *C*) allelic variants. Also, it has been shown association between allergenicity and protein variants encoded by the *Mal d 1.04* and *Mal d 1.06* genes (both located in linkage group 16). This association was confirmed for ten apple varieties (Golden Delicious, Priscilla, Ingrid Marie, Cox, Jonathan, Red Delicious, Fuji, Discovery and parental varieties Prima and Fiesta) (Gao Z.S. *et al*, 2008).

The most of studies is focused on determination of the allergenic protein content or genome mapping and there are fewer investigations aimed to establish the allergen gene expression.

Thus, the aim of this study was to reveal allergenicity of different apple varieties, widely presented at the market in Republic of Moldova through estimation of transcriptional activity of some *Mal d 1* allergen genes in peel and pulp.

MATERIAL AND METHOD

Biological material. Samples of pulp and peel from seven varieties widely presented at the market in Republic of Moldova (Richard (Gloster), Idared, Reinette Simirenko, Mantuaner, Jonathan, Golden delicious and Florina) have been studied. Collected samples were frozen in liquid nitrogen and stored at - 80°C until RNA extraction.

RNA extraction. Total RNA was extracted using CTAB buffer (2 % CTAB, 20 mM EDTA, 1,4 M NaCl, 100 mM TrisHCl pH 8.0, 2 % PVP K-90, 1 % β-mercaptoethanol) according to Bonghi C. *et al* (1992).

DNase treatment and cDNA synthesis. Obtained samples were treated with DNase I (Thermo Scientific) and were used for reverse transcription with RevertAid RT Reverse Transcription Kit (Thermo Scientific) supplied with Oligo(dT)18 primer (Thermo Scientific). Reactions were carried out according to manufacturer instructions. Samples were equalized by the cDNA amount.

Gene expression analysis. Gene expression of four allergen gene clusters (*Mal d 1.01, Mal d 1.02, Mal d 1.03* and *Mal d 1.04*) was studied using RT-qPCR in DT-96 termocycler (DNA technology, Russian Federation). Each reaction was performed in 15 µl using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) with 0.3 µM of each primer and 360 ng of cDNA. The cluster specific primers and Real-Time conditions were similar with those used by Botton A. *et al* (2008).

Every amplification was performed in three replicates. For normalization of the target gene expression the values of ubiquitin transcription activity were used (DQ438989).

Relative expression was determined according Livak K.J. and Schmittgen T.D. (2001). Specificity of amplification was confirmed by melting curve analysis, followed by 1.2 % agarose gel electrophoresis in TAE buffer.

Statistical analysis was performed according to Dospekhov B. (1985).

RESULTS AND DISCUSSION

Different values of gene expression were established for Mal d 1 clusters in seven apple varieties used in study.

The maximum values of transcript accumulation were attested for *Mal d 1.01* (from 1.5 to 16 conventional units (c. un.). *Mal d 1.03* and *Mal d 1.02* had intermediate expression levels. *Mal d 1.04* had minimal transcriptional activity values, which in most cases were very low or undetectable.

For *Mal d 1.01* the highest values of expression were detected in pulp and peel of Golden delicious, followed by Mantuaner and Florina (only pulp). In these varietes transcriptional activity of *Mal d 1.01* varied from 16 c.un. (pulp of Golden delicious) to 1.97 c.un. (peel of Florina). Minimal values of *Mal d 1.01* gene cluster expression were established in old German variety Richard (Gloster) – 1.5 and 1.67 c.un. in peel and pulp respectively *(figure 1).*

Values of *Mal d 1.02* gene expression are ranged from 0.0025 c.un. in pulp of Richard (Gloster) variety to 0.0847 c.un. in pulp of Idared variety. High values of transcriptional activity of *Mal d 1.02* were observed in pulp of Florina and Jonathan varieties. The lowest values were detected in old varietes Richard (Gloster), Reinette Smirenko and pulp of Golden delicious *(figure 1b).*

The *Mal d 1.03* cluster in all cases showed higher values of expression in peel of studied apple varieties than in pulp.

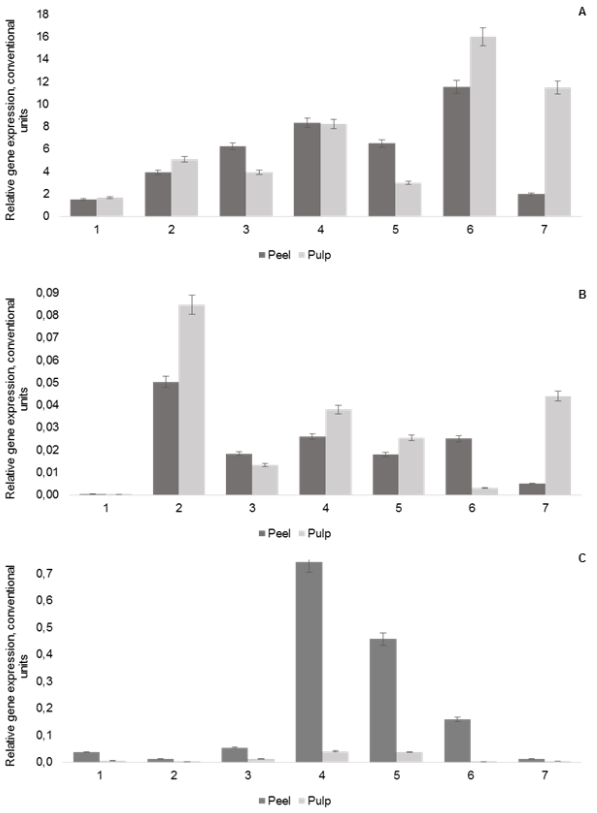

Figure 1 **Expression profile of *Mal d 1.01* (A), *Mal d 1.02* (B) and *Mal d 1.03* (C) gene clusters in the peel and pulp of different apple varieties. 1 – Richard (Gloster), 2 – Idared, 3 – Reinette Simirenko, 4 – Mantuaner, 5 – Jonathan, 6 – Golden delicious, 7 – Florina**

The most abundant presence of this transcript was detected in Mantuaner, Jonathan and Golden delicious peel *(figure 1C)*. Thus, the expression of this gene showed the genotype and tissue specificity.

The *Mal d 1.04* gene cluster mostly showed undetectable level of transcriptional activity. The highest value of expression was detected in peel of Mantuaner variety (0.000183 c.un.). Other varieties, such as Reinette Simirenko, Golden delicious and Florina, showed detectable values mostly in peel (data not shown).

Low transcriptional activity of *Mal d 1.04* gene was ascertained by the Botton A. *et al*, 2008, who analyzed gene expression of 12 genes encoding different isoforms of allergen proteins Mal d 1, Mal d 2, Mal d 3 and Mal d 4 in 15 apple varieties. Except *Mal d 1.04* gene, which expression varied slightly dependent on the genotype, all other genes showed different expression profiles.

The gene expression profiles of *Mal d 1* gene cluster varied considerably between studied genotypes. According to obtained data, there were established highly allergenic and hypoallergenic varieties. Thus, similarly with other studies (Puehringer H.M. *et a*l, 2003; Botton A. *et al*, 2008) Golden delicious was one of the most allergenic cultivars. Other varieties with high values of *Mal d 1* transcript accumulation were Florina and Mantuaner. In contrast to these varieties, old German variety Richard (Gloster) showed the lowest values of transcriptional activity for studied genes.

Also, it could be mentioned that Florina variety showed higher expression values for different allergen genes, including *Mal d 1*, in studies of Pagliarani G. *et al*, 2009, who assessed the level of allergen gene expression in association with genotype, tissue type and stage of fruit ripening.

Reinette Simirenko and Jonathan varieties showed higher values of *Mal d 1* allergen genes expression in peel than in pulp. Thus, it could be recommended to consume these cultivars without peel.

The obtained data and those reported by other authors demonstrate that allergenic properties of apple varieties differ very much and mechanisms which ensure these differences are not clearly understood.

Considering that in apples exist four main classes of allergenic proteins, it is important to extend this study to other genes and encoded proteins, which will be the aim of our further studies.

CONCLUSIONS

Expression analysis of *Mal d 1.01*, *Mal d 1.02*, *Mal d 1.03* and *Mal d 1.04* genes in apple peel and pulp allowed to establish varieties with high (Golden delicious, Florina and Mantuaner) and low (Richard (Gloster)) transcripts accumulation values and to suggest some recommendations regarding the apple consumption.

In contrast to other three studied genes (*Mal d 1.01*, *Mal d 1.02* and *Mal d 1.04*) the *Mal d 1.03* gene expression was tissue specific with higher values in peel than in pulp of all analyzed varieties.

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