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PEDIGREE INFORMATION SHARED IN APPLE ALLERGENS TRANSCRIPTIONAL ACTIVITY

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Apples are popular fruit worldwide thanks to their many tastes and a nutritional value. On the other side, they share the allergenic epitopes of Mal d 1 allergen that is relevant in food allegies. Sharing the genetic information of the origin accelerated the crossbreeding of world-class genotype in terms of the genetic resource of different apple species such as McIntosh, Jonathan, Golden Delicious, Granny Smith or Cox´s Orange Pippin. Among different characteristics, identification of genotypes with risk and perspective allergen potential should be focused on, in order to avoid apple disqualification in increasing food allergy. Here, based on own Mal d 1 gene expression analysis, 75 from 98 analysed varieties were directly linked to the common pedigree by 10 selected founding apple varieties - Golden Delicious, Jonathan, Clivia, Prima, Lord Lambourne, Rubín, Vanda, Cox´s Orange Pippin, Championa and Topas. In selected founding apple varieties we focus on genotypes from 2nd intergeneration (1870-95) to 5th intergeneration (2005-15). Expression data for of Mal d 1 recognized correlation between isoforms Mal d 1.02, Mal d 1.08 and selected founding apple varieties.

Keywords: apple, generation, Mal d 1 isoforms, real-time PCR, pedigree

INTRODUCTION

The founding apple variety is a world-class genotype in terms of the genetic resource of the species (McIntosh, Jonathan, Golden Delicious and Red Delicious in the Americas; Golden Delicious, Red Delicious and Granny Smith in the Western Pacific; Jonathan and Red Delicious in Western Europe; Cox´s Orange Pippin in the British Isles; Cox´s Orange Pippin and McIntosh in Eastern Europe (NOITON and ALSPACH, 1996). Due to their organoleptic properties, the proportion of these founding apple varieties has increased by more than 20% in recent decades,

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which logically also increased the probability of inbreeding future genotypes (NOITON and ALSPACH, 1996; BLAZEK and PAPRSTEIN, 2014). However, some indicators do not yet show such a trend (GROSS *et al*., 2014; ANASTASIADI *et al*., 2017). This is related to efforts to maintain the variability of current cultivars and the different prioritization of cultivars by consumers and breeders at national levels (NOITON and ALSPACH, 1996; GUARINO *et al*., 2006; GARKAVA-GUSTAVSSON *et al*., 2008; PATZAK *et al*., 2012; BLAZEK and PAPRSTEIN, 2014; LIANG *et al*., 2014; FERREIRA *et al*., 2016; LASSOIS *et al*., 2016; MARCONI *et al*., 2018; DENARDI *et al*., 2019). From the 1970s, breeding began to focus on the nutritional and agronomic value of fruits (PATZAK *et al*., 2014; ANASTASIADI *et al*., 2017). LOSSOIS *et al*. (2016) suggested that empirical selection of genotypes by pre-war growers (taste, color, size) could cause significantly greater damage to the integrity of the species' genetic resource than post-war selection breeding for resistance, allergenicity, or food focus (PATZAK *et al*., 2014; ANASTASIADI *et al*., 2017).

The topic of the study is based on the fact that the founding apple varieties and their genetic informations are shared in genotypes, breeding lines or ancestors. Genetic information from the ancestral genotype Ribston Pippin, through the genotypes Cox´s Orange Pippin, James Grieve, Greensleeves, Tuscan-Bolero, enters the variety Sonet in an indefinite construction. At the same time, the genetic information in a different construction enters the genotype Sonet also through the genotypes James Grieve, Lord Lambourne, Rubín, Topaz; or the genotypes Lord Lambourne, Vanda, Topaz (BARIC *et al*., 2020). At present, a large proportion of genotypes obtain genetic information of identical origin. The founding apple varieties Golden Delicious and Lord Lambourne were used to obtain the founding apple variety Rubín, the founding apple varietys Golden Delicious and Cox´s Orange Pippin participated in obtaining the founding apple variety Champion, and the founding apple varietys Rubín and Vanda were used to obtain the founding apple Topas. The founding apple varietys Golden Delicious and Jonathan, Golden Delicous and Clivia, Golden Delicious and Topas, Rubín and Clivia, Ruby and Prima, Jonathan and Champion, and Jonathan and Lord Lambourne have in common not only ancestors but also descendants. The connection between the founding apple varietys can also be found through some varieties (the founding apple varieties Rubín + Prima - varieties Priscilla and Elstar, the founding apple varietys Rubín + Vanda - varieties Bohemia and Rubinola, the founding apple varietys Golden Delicious + Vanda - variety Pink Lady, the founding apple varietys Topas + Champion - ÚEB genotype 1200/1). All these genotypes are well known and popular among consumers and growers but share genetic information of a few ancestral genotypes. In humans, inbreeding has the effect of genetic disorders, which are passed on to future generations with serious disorders. Whether it works that way in the plant kingdom goes by the way for draining food resources.

The main allergen of apples is the Mal d 1 protein with a size of 17.5 kDa, which belongs to the group of PR-10 proteins. PR-10 transcription is conditioned by various types of stress (MARI *et al*., 2005), and these proteins are responsible for defense responses in pathological conditions (VANEK-KREBITZ *et al*., 1995). The amount of transcript depends mainly on the variety of apple tree, but also on biotic and abiotic factors, conditions and length of storage (FERNANDES *et al*., 2013). In recent years, the chemical composition of the varieties has also been considered as one of the most important factors influencing the allergenicity of the variety. It was found that a higher content of polyphenols in fruits is directly related to reduced

allergenicity (KSCHONSEK *et al*., 2019). The reference structure of Mal d 1 was first described by a DNA sequence cloned from the cultivars 'Granny Smith' (isoform Mal d 1.0101) and 'Golden Delicious' (isoform Mal d 1.0102) and was identified down to the amino acid sequence level (PAGLIARANI *et al*., 2013; PUEHRINGER *et al*., 2003). Mal d 1 mRNA has been shown to be highly expressed in mature friuts and old leaves, while expression in young leaves has been reported at low levels, but may have been upregulated after exposure to various abiotic and biotic stressors (PUHRINGER *et al*., 2000). Isoform Mal d 1.02 was by far the most abundant expressed isoform in apple fruits, followed by Mal d 1.01 and Mal d 1.03. A similar distribution profile of transcript isoforms was observed in flowers, but at an expression level of several magnitudes, similar to young leaves. In contrast, Mal d 1.01 was predominantly expressed in vegetative tissues with the highest accumulation in mature leaves, medium expression in roots and low expression in young leaves. A comparable pattern, but with reduced transcription levels, was observed for Mal d 1.02 and Mal d 1.03, except for the latter, almost similar values were measured for mature leaves and roots (PUHRINGER *et al*., 2003). Patients allergic to birch pollen are often also hypersensitive to some proteins formed by various types of fruit or vegetables (GEROLDINGER-SIMIC *et al*., 2011). Birch pollen-related food allergies result from an initial sensitization to the major birch allergen (Bet v 1) and a subsequent immunological response of IgE antibody specification to homologous proteins in food. One of the most important cross-reactions is the immune response to apple consumption in the form of antibody formation, which affects >70% of patients allergic to birch pollen (BALLMER-WEBER, 2015).

The aim of the study was the analysis of changes in the expression of Mal d 1 allergen among apple varieties and its association with intensive breeding of a selected apple fouding varieties.

MATERIALS AND METHODS

Biological material of mature varieties *M. domestica* Borkh. (a total of 98 genotypes) was taken from a private orchard (Žilina District, Slovakia) in one day and stored at -20 °C. All the orchard has the same environmental conditions and the same agrotechnology was applied. A total of ten individual fruits were collected from every variety and biological triplicates were used for analysis. Total RNA was isolated using the GeneAll® RibospinTM Seed/Fruit Isolation System. After determining the quality and quantity of RNA in the samples, total RNA was transcribed into cDNA using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase® (Thermofisher Scientific), and the samples were then standardized to the same concentration. The Mx3000P qPCR System (Agilent Technologies) + SybrGreen DyNAmo flash master mix was used to measure Mal d 1 expression in transcriptomic assays. The primers used in the analyzes were designed by PAGLIARANI *et al*. (2013). The time-temperature regime of the reactions was as follows: initiation at 95 °C, 7 min; 45 cycles: denaturation: 95°C (30 s), annealing: 60-66°C (15 s), polymerization: 72 °C (15 s); fluorescence read after polymerization. This was followed by denaturation of the PCR products at 95°C for 60 s and melt analysis at 70°C - 95°C and fluorescence readings at a temperature change of 0.1°C. The concentration of primers in the reactions was 300 - 900 nM and the amount of cDNA was 0.5 - 1 µL depending on the measured isoform/isotype. The individual data sets were unified with a Granny Smith as variety calibrator. Testing of relationships within the pedigree consisted of the formation of clusters, both belonging to the founding apple variety, regardless of belonging to the founding

apple variety, and across the pedigrees (at least 5 genotypes in the cluster). 36 clusters of breeding lines in 21 pairs and 24 clusters of breeding generations in 19 pairs were tested. Control of the magnitudes of variations between the pairs of clusters of the respective Mal d 1 allergen isoforms, based on the variance (s) in a forward or inverse ratio (sd1, sd2) in the range of 0.5-3.0 of this ratio, preceded the choice of t-test ($P < 0.05$).

RESULTS

Structure of pedigree

The key point in classifying individual genotypes into a specific generation is dating of origin. Of the 98 monitored genotypes, 56 managed to be placed in a common pedigree through 10 so-called the founding apple varietys (Cox´s Orange Pippin, Jonathan, Golden Delicious, Lord Lambourne, Clivia, Prima, Vanda, Rubín, Champion and Topaz). It is a term denoting a genotype with a sufficiently wide spectrum with at least 5 monitored descendent genotypes of apple trees. In addition to them, other monitored genotypes are linked to the common pedigree, but in conflict with the criteria. The first is that the genotype must be directly linked to the pedigree through another monitored genotype. The second is that the genotype must not act as a transgenerational hybrid. And the last criterion is that in the case of a hybrid, the genotypes used to obtain it must share one generation. Based on this, the monitored genotypes form the following generations and intergenerations. 1st generation (1670-1770), 1st intergeneration (1770-1795), 2nd generation (1795-1870; Winesap, Cox´s Orange Pippin, Jonathan, Granny Smith), 2nd intergeneration (1870-1895; Red Delicious, Golden Delicious), 3rd generation (1895-1930; Lord Lambourne, Linda, Spartan, Spencer, Alkmene, Mutsu), 3rd intergeneration (1930-1945; Gala, Melrose, Akane, Fuji, Jonagold, Maigold, Freyberg), 4th generation (1945-1970; Braeburn, Rubin, Spigold, Pinova, Gloster, Prima, Ambrosia, Delcorf, Pikant), 4th intergeneration (1970-1995; Ligol, Fiesta, Pink Lady, Bolero, Champion, Sentima, Santana, Florina, Kiku, Delorina, Melodie, Amber, Jonalord, Delor, Bohemia, Selena, Vanda, Angold, Ecolette, HL 189, Kanzi, Primadela, Viktoria, Waltz), 5th generation (1995-2005; Resista, Rubinola, Topaz, Dulcit, Aneta, Goldstar, Rubin Step, Biogolden, Blanik, Crimson Snow, Dalinco, Funy, HL 782, Lotus, Product, Paradise), 5th intergeneration (2005-2015; Sonnet, Rozela, Sirius, Meteor, Opal, Heliodor, Rucla, Tab or, Carnival, Admiral, Lipno, Modi, Orion, Shalimar).

If the founding apple variety Golden Delicious leans into the 2nd generation (-1870), 6 genotypes from its breeding lines will reach the 3rd generation and one into the 4th generation. If the founding apple variety Golden Delicious appears in the 3rd generation (1895-) in its breeding line, 12 genotypes will get into the 4th generation (including the founding apples Rubín and the Champion) and 2 genotypes into the 5th generation. The Mutzu genotype would be located in the same generation as the founding apple variety Golden Delicious and up to 10 genotypes would be obtained by transgenerational hybrids (4th intergeneration - 3 genotypes, 5th generation - 3 genotypes, 5th intergeneration - 4 genotypes).

The Lord Lambourne variety is a 3rd generation variety. 2 varieties of this breeding line are in the 4th generation (including the Ruby variety) and 1 variety is a transgenerational hybrid. All varieties of the Rubín (4th generation) breeding line follow seamlessly from the Golden Delicious and Lord Lambourne breeding lines. In the 5th generation there are 8 of them

(including the Topas variety) and in the 6th generation 5 varieties. The Topas variety is a variety of one generation (5th) and 5 varieties of the 5th intergeneration would thus appear as varieties of the 6th generation in its breeding line. The mentioned variety Topas is also part of the breeding line of the variety Vanda (in the position of the variety of the 4th generation). To obtain another 3 varieties, the Vanda variety could figure not only in the position of the 4th generation variety (-1970), but also the 5th generation (1995-).

Another founding apple variety of one generation is Jonathan (2nd generation). Only 5 genotypes in the 3rd generation of this breeding line were not obtained by transgenerational hybrids. The remaining 8 genotypes range between 4th intergeneration and 5th intergeneration. There were 2 transgenerational hybrids (including the founding apple variety Champion) and 2 3rd generation genotypes in the Cox´s Orange Pippin founding line (2nd generation). The founding apple variety Champion is the second youngest founding apple with 2 genotypes as transgenerational hybrids, while 3 genotypes reached the 5th generation.

From the position of the 4th generation genotype, the founding apple variety Clivia participates in obtaining 4 genotypes in the 5th generation. The last of the founding apple varietys is Prima (from the 4th generation). There are 6 genotypes in the 5th and 1 genotype in the 6th generation in its breeding line.

Of the 27 genotypes monitored, 8 were without generational placement, 8 genotypes with generational placement without a link to the founding apple trees (1 in 2nd generation, 1 in 3rd generation, 2 in 4th generation, 1 in 4th intergeneration and 3 in 5th generation) and 10 genotypes with generational location and also linked to the founding apple varietys (Granny Smith - 2nd generation; Red Delicious, Linda, Spartan - 3rd generation; Kiku, Braeburn, Gloster - 4th generation; Santana, Bolero - 4th intergeneration and Crimson Snow - 5th generation).

After summarizing the breeding lines of all founding apple varietys, 1 genotype appears in the second generation, 11 genotypes in the 3rd generation, 13 genotypes in the 4th generation, 21 genotypes in the 5th generation, 11 genotypes in the 6th generation and 19 transgenerational hybrids. From the included genotypes, the mentioned genotypes Rucla, Gala, Rozela, Karneval, Lipno and Tabor appear simultaneously in two generations. Other genotypes are transgenerational hybrids of founding apple trees (indicated in parentheses) Resistance Opal, Shalimar, Heliodor, Syrius (all for founding apple variety Golden Delicious), Champion (for founding apple variety Cox´s Orange Pippin), Funy (for founding apple variety Jonathan) and Blanik (to the Florina apple tree, to the Jonathan line). The genotypes Jonalord and Amber are double transgenerational hybrids (both to the founding apple varietys Golden Delicious and Jonathan).

Expression activity of Mal d 1 isoforms

Apple tree genotypes are represented by the expression of target allergen genes (Mal d 1) in the 3-6. generation of apple genotypes (Figures 1-4). When comparing 3rd generation and 4th generation, isoforms are grouped into 3 groups (A - Mal d 1.03F, 1.03G, 1.06A and 1.07; B - Mal d 1.01, 1.02, 1.03D, 1.06B, 1.06C, 1.08 , 1.11A and 1.13A; C-Mal d 1.03C and 1.03E). None of the isoforms showed a statistically significant difference (t-test: 0.247-1.025 at a critical value of 1.706-1.782). When comparing the 4th generation and the 5th generation, there are only 2 groups (A - Mal d 1.01 and 1.07; B - Mal d 1.02, 1.03C, 1.03D, 1.03E, 1.03F, 1.03G, 1.06A,

1.06B, 1.06C, 1.08, 1.11A and 1.13A). This time, at a critical value of t-test (1.686-1.753), a statistically significant difference was detected in two cases; Mal d 1.02 and 1.06A (t-test: 1.702 and 2.053). The difference in the case of the Mal d 1.02 isoform occurred when comparing the 5th generation and the 6th generation, where all isoforms formed a single group. The t-test value in this case was -1.923 at a critical value of 1.686. In all figures, the isoforms Mal d 1.01 and 1.02 protrude at the lower limit and Mal d 1.08 protrudes at the upper limit. Dots closer to the lower limit show higher expressed genotypes and dots closer to the upper limit with lower expression. The highest density of genotypes with higher expression is in the genotypes Golden Delicious, Champion, Melody, Funy, Jonagold, Jonaprince, Gala, Evelina, Pinova, Ligol2, Delor, Tabor, Bohemia, Rozela1, Rozela2, Admiral, Rubinola, Lipno, Fiesta, Resistent Opal and Souet on isoforms Mal d 1.01, 1.02, and in a few cases also on isoforms Mal d 1.06A and 1.08 (Figures 1-4). Assigning specific genotypes to the group of genotypes with low Mal d 1 expression is complicated by extreme values for Mal d 1.03C and 1.06C isoforms (Fig. 3) in the 4th generation of apple genotypes.

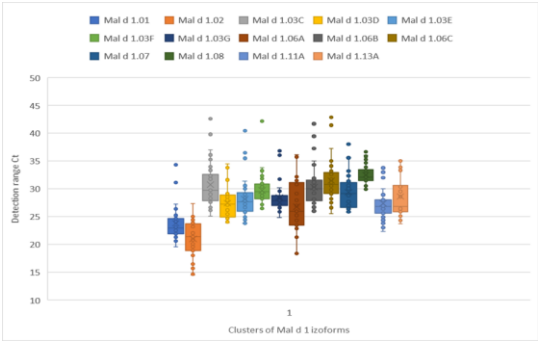

Figure 1. Comparison of expression among Mal d 1 isoforms of the 3rd generation genotypes

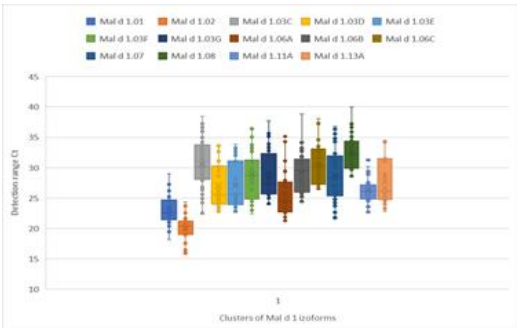

Figure 2. Comparison of expression among Mal d 1 isoforms of the 4th generation genotypes

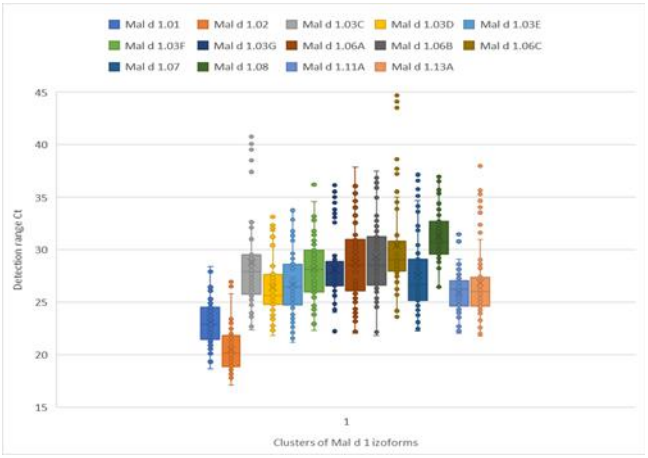

Figure 3. Comparison of expression among Mal d 1 isoforms of the 5th generation genotypes

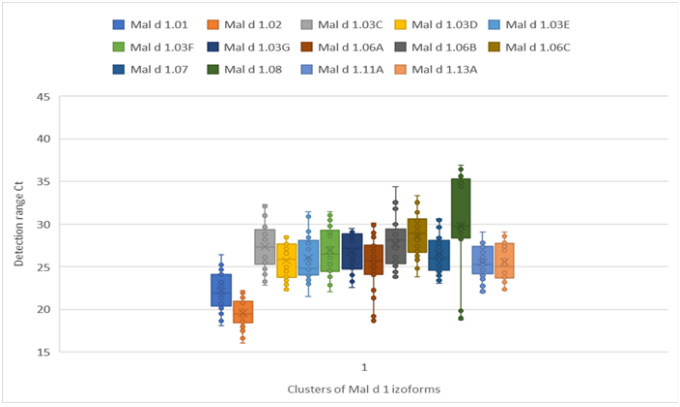

Figure 4. Comparison of expression among Mal d 1 isoforms of the 6th generation genotypes

The detection range of values in the 3rd generation of genotypes has the smallest density on the Mal d 1.06A and 1.08 isoforms, but the largest on the Mal d 1.06B isoform. The diagram density is set between 20-35 (90.48%) and 25-35 (73.08%). Isoforms Mal d 1.02 and 1.13A with the smallest range in the 4th generation (density between 20-35 (92.54%) and 25-35 (62.38%) and isoform Mal d 1.08 with the largest in the 6th generation (density between 20-35 (90.24%) and 25-35 (55.95%)) is also recognizable. The 5th generation of genotypes shows constant ranges among all Mal d 1 isoforms (density between 20-35 (90.29%) and 25-35 (66.67%)) (Figures 1-4). Meanwhile, the density of the wider range peaks at about 90% (expected), in the narrower range it is disproportionate to the generation, the highest density has the 3rd generation with 11 genotypes, the lowest in the 6th generation with the same number of genotypes and the 5th generation with the largest number of genotypes, only 66.67% The point of interest, which claims that there is a suspicion of correlation, has failed. Density in detection range from 20-35

to 25-35 in the 3rd generation on isoforms Mal d 1.01, 1.02 drastically decline, moderate on isoforms Mal d 1.06A, and slightly on isoforms Mal d 1.03D, 1.03E, 1.11A and 1.13A (Table 1). In the 4th generation on isoforms Mal d 1.01, 1.02 continues drastic decline. The moderate decrease in density from 20-35 to 25-35 on the Mal d 1.06A isoform was accompanied by the Mal d 1.03D and 1.03E isoforms. A slight decrease in Mal d isoforms 1.11A and 1.13A was recorded for Mal d isoforms 1.03F, 1.03G and 1.07 (Table 1). Only 41.33% density on the Mal d 1.02 isoform is an unexpected result of the expression of this Mal d 1 isoform. The remaining isoforms showed a similar density range as in previous generations (Table 1). Finally, the 6th generation of genotypes provides an even lower density on the Mal d 1.02 isoform (36.67%), suggesting a correlation in Mal d 1.02 isoform expression and genotyping based on dating, similar to the correlation with Mal d 1.02 isoform (Table 1, Figure 4).

Table 1. The density of genotypes of gene Mal d 1 expression in the detection range Ct.

Isoform of allergen	Detection range of CT values (generation)							
	20-35 (3rd)	25-35 (3rd)	20-35 (4th)	25-35 (4th)	20-35 (5th)	25-35 (5th)	20-35 (6th)	25-35 (6th)
Mal d 1.01	97.44 %	20.51 %	93.33 %	17.78 %	94.67 %	16.00 %	86.67 %	10.00 %
Mal d 1.02	69.23 %	12.82 %	68.89 %	0.000 %	41.33 %	4.000 %	36.67 %	0.000 %
Mal d 1.03C	87.18 %	87.18 %	84.44 %	82.22 %	88.00 %	74.67 %	100.0 %	76.67 %
Mal d 1.03D	100.0 %	74.36 %	100.0 %	55.56 %	100.0 %	69.33 %	100.0 %	53.33 %
Mal d 1.03E	92.31 %	79.49 %	100.0 %	64.44 %	100.0 %	72.00 %	100.0 %	46.67 %
Mal d 1.03F	97.44 %	97.44 %	91.11 %	64.44 %	98.67 %	81.33 %	100.0 %	66.67 %
Mal d 1.03G	92.31 %	89.74 %	93.33 %	75.56 %	94.67 %	81.33 %	100.0 %	66.67 %
Mal d 1.06A	84.62 %	48.72 %	97.78 %	40.00 %	94.67 %	77.33 %	90.00 %	56.67 %
Mal d 1.06B	89.74 %	89.74 %	97.78 %	84.44 %	88.00 %	84.00 %	100.0 %	86.67 %
Mal d 1.06C	87.18 %	87.18 %	95.56 %	95.56 %	84.00 %	80.00 %	100.0 %	93.33 %
Mal d 1.07	92.31 %	92.31 %	93.33 %	71.11 %	94.67 %	70.67 %	100.0 %	73.33 %
Mal d 1.08	84.62 %	84.62 %	80.00 %	80.00 %	90.67 %	90.67 %	50.00 %	50.00 %
Mal d 1.11A	100.0 %	79.49 %	100.0 %	73.33 %	100.0 %	66.67 %	100.0 %	53.33 %
Mal d 1.13A	92.31 %	79.49 %	100.0 %	68.89 %	94.67 %	65.33 %	100.0 %	50.00 %

DISCUSSION

Currently, pedigree relationships in apple gene resources are most commonly identified and verified through SSR molecular marker analyzes, which were initially performed at the regional level only (GUARINO *et al*., 2006; GARKAVA-GUSTAVSSON *et al*., 2008; PATZAK *et al*., 2012; LIANG *et al*., 2014; FERREIRA *et al*., 2016; LASSOIS *et al*., 2016; URRESTARAZU *et al*., 2016; LARSEN *et al*., 2017). Due to the lack, inaccuracy or incompleteness of historical sources on the geographical distribution of apple cultivars and genotypes, it is extremely difficult to geogenetically trace genotyping (LOSSOIS *et al*., 2016; URRESTARAZU *et al*., 2016). Redundancy of genotypes in different countries also makes tracing difficult. In addition, genotypes with similar profiles appear under different designations or, conversely, different profiles under the

same designation (EVANS *et al*., 2010; LASSOIS *et al*., 2016; URRESTARAZU *et al*., 2016). The lack of information is an attempt to verify by TTT (true-to-type) approach, especially in cases where the pedigree relationship is not clearly defined (chance seedling, crabapple, unregistered genotypes, genotypic intermediates, species genotypes of the genus *Malus*) or innovative approaches "*machine learning methods*", RNA inteference (GILISSEN *et al*., 2005; EVANS *et al*., 2010; SALVI *et al*., 2014; ANASTASIADI *et al*., 2017).

Chronological generation of genotypes into generations such as that performed in this study is not a traditional approach but correlate with previous studies (BARIC *et al*., 2020; MURRANTY *et al*., 2020). Dating makes it possible to place changes in genetic information revealed by molecular methods over time. One example is the variety Red Delicious (1870), which was created after people selected apples with a rough skin and bred them together for several years. This extended shelf life to the detriment of future genotype variability (KRINOS and HIDAYAT, 2019). Red Delicious is a genotype of the interface of the 2nd generation (1795-1870) and the 2nd intergeneration (1870-1895) which means that genotypes with a thin shell are dated to this period (McIntosh, Golden Delicious, Red Delicious, Granny Smith) and to the interwar one (3rd intergeneration - 1930-1945) (Jonagold, Fuji, Empire, Braeburn, Gala). Variety Jonagold was a direct cross between the varieties Jonathan and Golden Delicious (1943) and tends to have fairly large apples, suitable for both baking and eating. The Jonagolds are a remnant of the pre-genomic era and were created by applying pollen from Jonathan apple trees to red delicious flowers of variety Golden Delicious. Another example of a strategy is to maintain genetic variability by inserting the *Vf* gene, which was obtained from *M. floribunda* Sieb clone 821. and later anchored in *M. floribunda* type F3 genotypes, (Golden Delicious × F2 26829-2-2), (Jonathan × F2 26829-2-2) or (Melba × (Wealthy × Starr)) (NOITON and ALSPACH, 1996). Its foundations were laid by the PRI (1945) Breeding Program (JANICK, 2000). Many genotypes of the apple tree Prima (1970) from the mentioned program inherit such a gene structure in the genome (observed genotypes mostly 5th generation - HL 189, Tabor, Ecolete, Selena, Rubinola, Rezista, Lipno). MURRANTY *et al*. (2020) published one of the largest pedigree analyzes SNPs enabled us to use simple exclusion tests based on Mendelian error counts to identify numerous parent-offspring relations and consequently reconstruct multi-generation pedigrees involving cultivars selected during several centuries using the AxiomRApple480K array (BIANCO *et al*., 2016). Their aim was to shed light on chance seedling, crabapple, unregistered genotypes, genotypic intermediates, species genotypes of the genus Malus, or to confirm or refute unverified pedigree relationships (Ribston Pippin > Cox´s Orange Pippin; Geheimrat Doktor Oldenburg × Cox´s Orange Pippin > Dukat; Grimes Golden > Golden Delicious; Borowitzky = Charlamowsky = Duchess of Oldenburg = Dutch Migninne = Reinette de Caux). A similar study was contributed by BARIC *et al*. (2020).

The expression of diferent Mal d 1 genes is induced by temperature, light, and water stress (BOTTON *et al*., 2008). During storage was observed an increase of the Mal d 1 transcripts, the highest values being obtained after 2 and 5 months of storage (SANCHO *et al*., 2006), facing abiotic stressors of storage (PUHRINGER *et al*., 2000). Thanks to this, the comparative analysis of the Mal d 1 expression has some limits. Here, all of the analysed varieties were from one orchard and collected in one day, what minimizes the effect of different environmental conditions and external factors. Variability between Mal d 1 isoforms shows a pattern of increasing and

decreasing gene expression during ripening of apple fruits (URBANOVÁ, 2021). The gene expression of Mal d 1.01 and 1.06A is higher in old apple cultivars than in new ones (SIEKIERZYNSKA *et al*., 2021). In cv. Golden Delicious, Idared, Jonagored or cv. Gala, the expression of Mal d 1.06A positively correlated with the fruit maturity and with the immunoreactivity of patients' sera. The expression of Mal d 1.01 was higher in the skin than in the flesh in old varieties. Among old varieties, the level of Mal d 1.06A expression was lower than of Mal d 1.01 in the average expression and in the skin. All these influences on gene Mal d 1 expression were consistent during cultivation and storage for all genotypes so the difference reflected on the ability of genetic information to adapt to unified conditions. For example, higher polyphenol content in fruits is directly related to reduced allergenicity (KSCHONSEK *et al*., 2019).

CONCLUSIONS

Mal d 1 allergen and its isoforms is a model for pollen sentitising food allergies. The rising knowledge about its varietal based differencies will help in the future to set up the personalized management of allergic patients towards the possibilities of consumption of this fruit. Here, ten founding apple varieties were combined into one pedigree collecting the information abou Mal d 1 allergen isoforms mRNA expression. In the 2nd generation 1 genotype is determined, in the 3rd generation 11 genotypes, in the 4th generation 13 genotypes, in the 5th generation 21 genotypes and in the 6th generation 11 genotypes. The intergenerational correlation in expression was not confirmed. Nevertheless, there were two isoforms of Mal d 1, where the expression in relation to the generations of apple genotypes showed signs of shifts (increase - Mal d 1.02, decrease - Mal d 1.08). The Mal d 1 allergen expression test could light up if this is a warning signal for centuries of crossbreeding side effects.

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PODACI O PEDIGREU UTVRĐENI U TRANSKRIPCIONOJ AKTIVNOSTI ALERGENA JABUKE

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Izvod

Jabuke su popularno voće širom sveta zahvaljujući svom ukusu i nutritivnoj vrednosti. S druge strane, dele alergene epitope Mal d 1 alergena koji je relevantan za alergije na hranu. Razmena genetskih informacija o poreklu se ubrzava ukrštanjem svetski poznatih genotipova jabuke (*Mcintosh, Jonathan, Golden Delicious, Granni Smith, Cok's Orange Pippin*). Potrebno je fokusirati se na identifikaciju genotipova sa rizičnim i perspektivnim alergenskim potencijalom kako bi se izbegla diskvalifikacija jabuke, koja povećava alergiju na hranu, u budućim generacijama genotipova. Iz biološkog materijala sorti *M. domestica* Borkh. (ukupno 98 genotipova) je izolovana ukupna RNK i transkribovana u cDNK. Na osnovu liste genotipova, 75 genotipova je direktno povezano u zajednički pedigre sa 10 unapred odabranih sorti jabuke (*Golden Delicious, Jonathan, Clivia, Prima, Lord Lambourne, Rubin, Vanda, Cok´s Orange Pippin, Champion, Topas*). Ispitani genotipovi formiraju 5 generacija i 5 međugeneracija. U ovom radu fokusirali smo se na genotipove od 2. međugeneracije (1870-95) do 5. međugeneracije (2005-15). Merenjem Mal d 1 ekspresije utvrđena je korelacija između izoformi Mal d 1.02, Mal d 1.08 i starijih generacija genotipova jabuke.

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