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EXPRESSION ACTIVITY OF ALLERGEN CODING GENES IN GRAPE VARIETIES USED FOR TOKAJ WINE PRODUCTION

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Vitis vinifera L. is adapted to a very variable range of climates but it mostly grows in the temperate regions of continental Europe. In the Slovak Republic, the Tokaj wine region is one of the territories, where wine production is concentrated. Grape is a popular fruit and when processed, it is used as musts, juices or wine. Some people may suffer from allergic reactions to grapes. Up to now, endochitinases, lipid-transfer protein, and thaumatin were identified as grape allergens. In this study, expression of chitinase and thaumatin allergen was analysed in the grapes of Tokaj region varieties – Hachat Lovelin, Tokay and Muskat Blanc. Expression changes were calculated by the delta delta Ct method. Expression differences of chitinase were found to be similar in these varieties. Thaumatin was found to be variable in its transcription.

Keywords: Tokaj region grape varieties, chitinase, thaumatin, expression

Vitis vinifera L., is one of the oldest cultivated plants all over the world. It is well adapted to a wide range of climates, but it grows especially well from the temperate Mediterranean regions to the continental areas in Central Europe. Grape fruit is widely consumed either directly or as wine. Western Europe is the world's biggest producer of grapes, mainly France, Italy and Spain are the major producers of wines (Pastorello et al., 2003). The health beneficial effects of grapes and wine are very well known due to their high nutritional value and unique phytochemical composition. *Vitis vinifera* is a major source of polyphenols, flavonoids, anthocyanins, phenolic acids, stilbenes, vitamins (A and C), minerals (phosphorus, calcium) and carbohydrates (Arora et al., 2016). It has recently been observed that moderate consumption of grapes or red wine has many health beneficial effects: anti-asthmatic, cardio protective, cytotoxic, anti-aging, hepatoprotective, anti-inflammatory, and antioxidant (Ahmad and Khan, 2012; Masani et al., 2012).

Many of grapes' biochemical and technological characteristics are based on climatic conditions where the plants grow. In this study, grape varieties from the Tokaj wine region were used. The Tokaj wine region is quite a unique one with the following characteristics. It consists of clay or loess soil on volcanic subsoil with sunny microclimate. Some specific grape varieties such as Tokay and Hachat Lovelin have been cultivated in the region for centuries and, together with Muskat Blanc are the only grape varieties that are planted in this region.

Despite many health protective and beneficial effects of *Vitis vinifera*, some people suffer from allergic reactions to this fruit. Major allergens of wine and grapes: 30-kd endochitinase 4A and 4B, 9-kd lipid-transfer protein (LTP) and 24-kd thaumatin were characterized and identified by Pastorello et al. (2003). The precise molecular mechanism of action of grape allergens has not been yet studied and therefore requires our attention and also the need to develop novel diagnostics methods and improve treatment management in this field. Chitinases are the most active protein components in causing wine turbidity (Falconer et al., 2010; Marangon et al., 2011). Chitinases derived from grapes are present in different isoforms (Marangon et al., 2011; Gazzola et al., 2012) and are tolerant to low pH in juice and wine as well as resistant to photolytic enzymes, as most of the pathogenic related proteins (Ferreira et al., 2001; Waters et al., 2005; Van Sluyter et al., 2015). Thaumatin was identified in tropical plant *Thaumatococcus danielii* Benth as a protein having sweet taste. A specific domain common to osmotin-like proteins and a kinase receptor of PR5-like proteins was described in its structure and grouped together as the base of the thaumatin-like protein family (Wang et al., 2011). These proteins are much diversified in their functions and were described to be involved in stress responses (Yan et al., 2017). Thaumatin-like protein structure was defined in grapes by Marangon et al. (2014) and physico-chemical parameters relevant for the haze formation mechanism were determined by these authors.

Beside the relevance in winemaking, thaumatin is defined as a minor grape allergen together with chitinases (Vassilopoulou et al., 2007). Its relevance is important, because grapes are consumed not only processed, but fresh, too, and some severe allergic reactions were reported in the case of grape consumption in the Mediterranean region before (Kalogeromitros et al., 2006). Grape allergy is

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clinically manifested by severe symptoms mainly in patients suffering from multiple allergies and preferentially to LTP (lipid transfer proteins) containing foods (Vassilopoulou et al., 2007) and an association with peach and cherry allergy was observed (Pastorello et al., 2003). Actually, there is no specific information about the differences on transcriptomic level for the allergens and their genes. In this study, expression of chitinase and thaumatin-like allergen was analysed in the grapes of three grape varieties planted in the Slovak Tokaj wine production.

Material and method

Biological material

Grapes of three varieties of *Vitis vinifera*, L. that are used for producing Tokaj wines were obtained in the season 2018 in the Slovak Tokaj wine region. Only grapes without any infection marks were used for further analysis. Fresh matured grapes of varieties Lipovina (syn. Hachat Lovelin, Lindenblütrige, Hárselevelű, Kerekes, Kereklevelű), Furmint (syn. Tokay, Mosler, Luttenberger, Weisslauber, Edler weisser Tokayer, Malnik, Furmint bianco, Bihari boros, Demjén, Formont, Furmin) and Muškát žltý (syn. Muškát Lunel, White Frontigan, Muskat Blanc, Muscat de Lunel, Muscat de Frontignan, Bárzsing, Fehér muskotály, Gelber Muskateller, Muskateller, Weisse Muskattraube, Muskatel menudo bianco, Moscato bianco) were harvested and after surface cleaning transported immediately to the laboratory where they were kept in -50 °C until further processing.

RNA extraction and cDNA synthesis

The total RNA was extracted using the GeneJet Plant RNA Purification Mini Kit (ThermoFisher) following the manufacturer´s instruction with a modification of the weight of homogenized tissue use. The obtained RNA concentration and A260/A280 nm ratios were determined by Implen Nanophotometer and the integrity of the RNA was checked in 1% agarose gels. cDNA synthesis was performed from 30 ng of total RNA using the Tetro cDNA synthesis kit (BIOLINE) with the oligodT primer.

Analysis of the expression of chitinase and thaumatin

A two-step protocol was used for both, chitinase and thaumatin expression analysis where the gene for actin (GenBank accession AY847627) was used as the internal control during qPCR. Amplification was performed using EliZyme Green MIX AddROX (Elizabeth Pharmacon) in Stratagene Mx3005P thermal cycler (Agilent). The following program was used for chitinase transcripts amplification: 95 °C for 2 minutes followed by 40 cycles of 95 °C for 10 seconds, 60 °C for 40 seconds, ended by dissociation curves analysis of amplified thaumatin products by heating the amplicon from 75 °C to the 95 °C. All the reactions were performed in triplicates. The thaumatin transcript analysis followed the PCR conditions of Žiarovská et al. (2019). Chitinase specific primers were designed on the basis of its sequence stored in the GeneBank under acession DQ267094 and thaumatin specific primers were designed on the basis of coding region of the genomic sequence stored in the GeneBank under accession AF227324.

Results and discussion

Expression profiles of chitinase and thaumatin were analysed in three Tokaj grape varieties – Hachat Lovelin, Tokay and Muskat Blanc. Actin was used as an internal control for the correction of sample-to-sample variation. The generated Cts of actin amplicons ranged from 18.30 (Hachat Lovelin) up to the 20.85 (Tokay). Dissociation curves of amplified actin products calculated during the melting procedure showed a single melting peak with melting temperature (Tm) of 81.8 °C.

Dissociation curves of amplified chitinase products showed a single melting peak with melting temperature (Tm) of 85.8 °C, indicating specific product. Control reactions of NTC (non-template control) generated clearly differentiated products. Cts of chitinase generated amplicons during the real-time PCR analysis varied from 29.70 (Hachat Lovelin) to 31.64 (Tokay). Expression changes of chitinase allergen were calculated by the delta delta Ct method (Livak and Schmittgen, 2001). Chitinase expression was found to be similar in the three analysed grape varieties. The Tokay grape variety was found as having the lowest activity in chitinase transcription activity, counted in 36 fold percentage decreasing when compared to the Hachat Lovelin variety and 63 fold percentage decreasing when compared to the Muscat Blanc variety. Muscat Blanc has the expression level of chitinase very similar when compared to the Hachat Lovelin with the increasing 0.21 fold change (Fig. 1).

Dissociation curves of amplified thaumatin products in the Slovak Tokay grape varieties calculated during the melting procedure showed a single melting peak with melting temperature (Tm) of 87.5 °C, indicating specific product. Control reactions of NTC generated clearly differentiated products. Thaumatin expression was found to be more variable in the three analysed grape varieties when compared to the expression of chitinase. Here again, the Tokay grape variety was found as to have the lowest activity in thaumatin transcription activity, counted in 100 fold percentage decreasing when compared to the Hachat Lovelin variety and 186 fold percentage decreasing when compared to the Muscat Blanc variety. Interestingly, Hachat Lovelin has the expression level of thaumatin 10 times higher when compared to the Muscat Blanc (Fig. 2).

Up to now, only a few studies have existed that analyse the expression activity of grape allergens. Thaumatin expression analysis was performed previously by Žiarovská et al. (2019) in grapes of four red varieties of *Vitis vinifera*, L. that were obtained in the season 2017 in the Sabo winery that belongs to the Malokarpatský wine region. Dornfelder was found to have the lowest activity in thaumatin-like gene activity, mainly when compared to Cabernet Sauvignon and Frankovka modrá. Alibernet, on the other side, has the expression level of thaumatin very similar when compared to Cabernet Sauvignon and Frankovka modrá.

Expression profiles of different thaumatin-like proteins were analysed by Yan et al. (2017) in the varieties Red Globe, Shang-24, Hunan-1 and Shuangyou under the inoculation of three different pathogens with the conclusion that the expression of this gene family is broadly influenced by *Botrytis cinerea*, *Elsinoe necator* and *Elsinoe ampelina*.

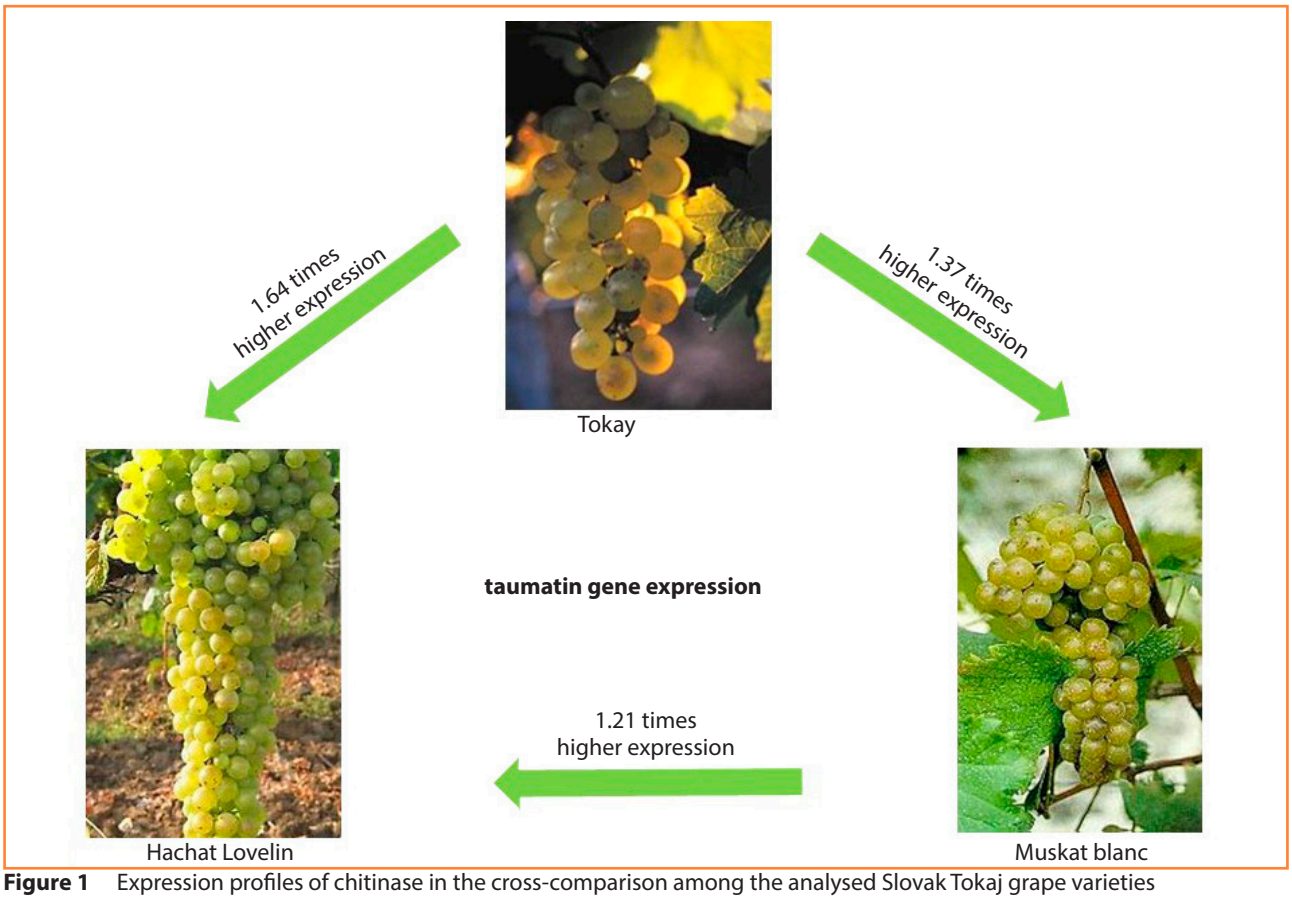

Figure 1 Expression profiles of chitinase in the cross-comparison among the analysed Slovak Tokaj grape varieties

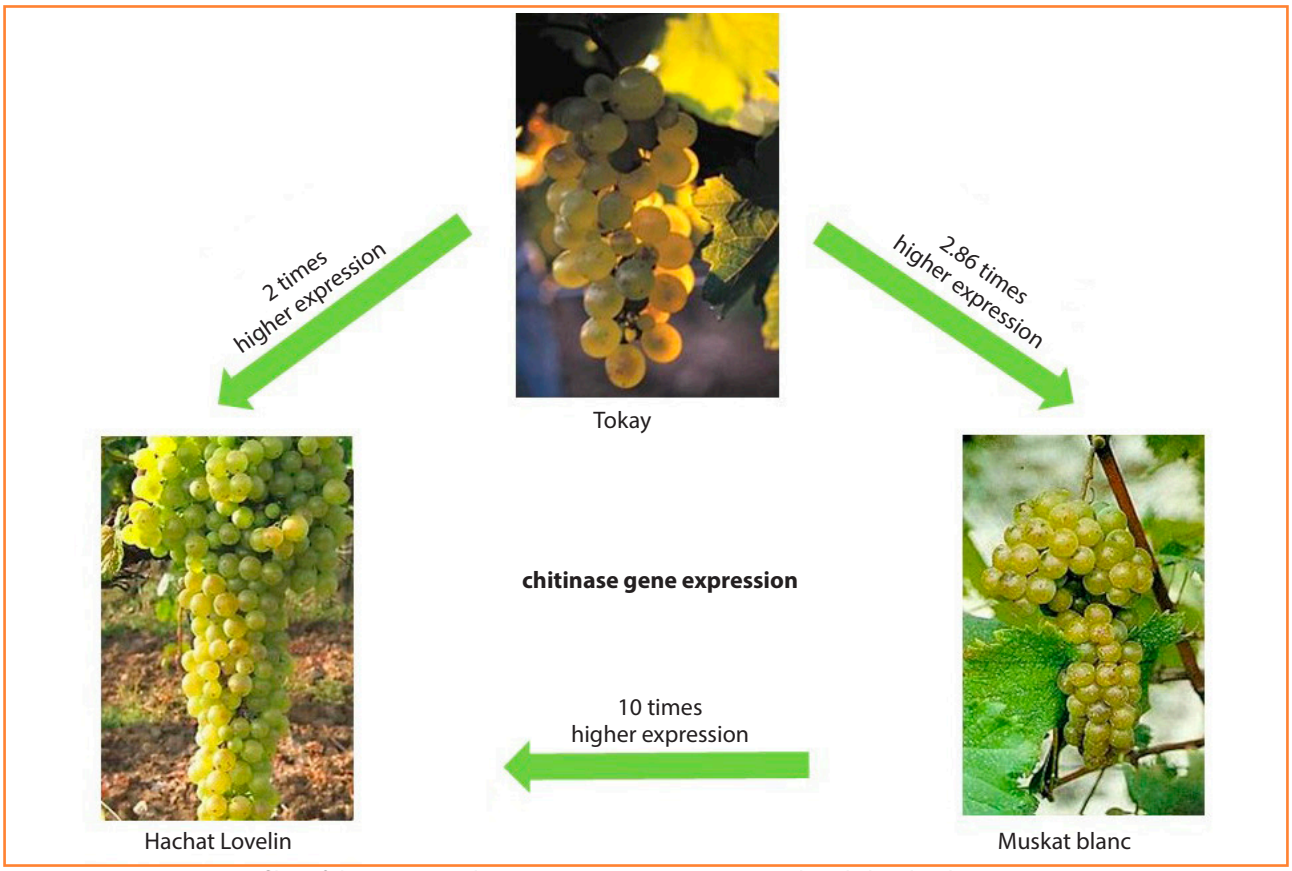

Figure 2 Expression profiles of thaumatin in the cross-comparison among analysed Slovak Tokaj grape varieties

Expression of thaumatin in grape is reported up to now to be affected by different panthogens. Subsequence of inoculation by anthracnose, powdery mildew and *Botrytis* was analysed in the sense of thaumatin-like genes expression in three different grape varieties by Yan et al. (2017) with the conclusion that different genes were increased in their expression following each of the inoculation pattern.

Conclusion

Chitinase and thaumatin are both minor allergens in grapes, but their persistence to wine and juice products is relevant to people suffering from grape allergy. Expression differences of chitinase and thaumatin in the Tokaj region grape varieties – Hachat Lovelin, Tokay and Muskat Blanc show that chitinase is similar in its expression in these varieties, but taumatin is much more variable in transcription. Muscat Blanc was found to have the highest level of thaumatin expression with the expression fold change of 10.

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