

EXPRESSION PATTERN OF THAUMATIN IN THE SELECTED RED VARIETIES OF *VITIS VINIFERA*, L.

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ABSTRACT

Vitis vinifera L. is a specie that is adapted to a very variable range of climates, from cold up to the desert one, but especially it grows in the temperate Mediterranean regions and continental areas of Europe. Grape is a widespread consumed fruit as well as processed to musts, juices or wine. The health beneficial effects of grapes and wine are very well known due to their high nutritional value and unique phytochemical composition. Despite many health protective and beneficial effects of *Vitis vinifera*, a part of population suffer to allergic reactions to this fruit. Allergens of wine and grapes are: endochitinases, lipid-transfer protein and thaumatin. Thaumatin is a protein having a sweet taste belonging to the PR5-like proteins. These proteins are very diversified in their functions and were described to be involved in stress responses and fruit ripening, but are expressed in healthy grape fruits in a constitutive manner and needn't to be expressed only as a answer to the stress. Thaumatin is a minor allergen in grape, but belonging to the suspected panallergens relevant to the food cross-allergy induction, its importance is quite high. Another importance of this protein is a technological one, as reported to aggregate in wine to form a visible haze unless removed prior to bottling. In this study, expression of thaumatin-like allergen was analysed in the grapes of selected varieties. Grapes of four red varieties of *Vitis vinifera*, L. were obtained in the season 2017 in the Sabo winery that belongs to the Malokarpatská wine region. Fresh matured grapes of varieties Alibernet, Cabernet Sauvignon, Frankovka modrá and Dornfelder were analysed. Expression changes of thaumatin was calculated by delta delta Ct method. Dornfelder was found as to have the lowest activity in thaumatin-like gene activity, mainly when comparing to the Cabernet Sauvignon and Frankovka modrá. Alibernet, on the other side, has the expression level of thaumatin very similar when comparing to the Cabernet Sauvignon and Frankovka modrá.

Keywords: *Vitis vinifera*, L.; thaumatin; expression; red varieties

INTRODUCTION

Vitis vinifera (Family *Vitaceae*), commonly known as grapes, is one of the oldest cultivated plants all over the world. It adapted to a vast range of climates, from the cold areas of Russia to the desert regions of California, especially it grows from the temperate Mediterranean regions to the continental areas in Central Europe. This fruit is widespread consumed either directly or as wine. The Western Europe is the world's biggest producer of grapes, mainly the France, Italy and Spain are the major producers of wine (approximately more than 16 millions tons per year) (Pastorello et al., 2003). Additionally, social involvement in grape harvesting and farming is very high in same areas of these states, with the participation of 20% to 30% of the local population (Brito et al., 2008). 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, cardioprotective, cytotoxic, anti-aging, hepatoprotective, anti-inflammatory, antioxidant (Cui et al., 2002; Bathomeuf et al., 2006; Ahmad and Khan, 2012; Masani et al., 2012). In addition, some studies were demonstrated, that grapes and red wine has anticancer properties. Resveratrol, belonging to the class of stilbenes, secondary metabolites produced by this plant, has showed a promising role as multidrug anti-cancer agent in cancer chemoprevention and treatment and also in treatment of neurological diseases (Varoni et al., 2016; Andrade et al., 2018).

Despite many health protective and beneficial effects of *Vitis vinifera*, a part of population suffer to allergic reactions to this fruit. Pastorello et al. (2003) characterized and identified the major allergens of wine and grapes: 30-kd endochitinase 4A and 4B, 9-kd lipid-transfer protein (LTP) and 24-kd thaumatin by mass spectrometry and amino acid sequencing. They also reported that LTP protein has a high rate of sequence

homology with peach and cherry LTPs proteins, which gives a molecular basis of allergen cross-reactivity (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 need to develop novel diagnostics methods and improve treatment management in this field.

Thaumatococin is a protein to be identified in tropical plant *Thaumatococcus daniellii* Benth and having a sweet taste. A specific domain common to osmotin-like proteins and a kinase receptor of PR5-like proteins was described in the structure and grouped together they are the base of thaumatin-like protein family (Wang et al., 2011). These proteins are very diversified in their functions and were described to be involved in stress responses (Yan et al., 2017).

Pathogenesis-related proteins are expressed in healthy grape fruits in a constitutive manner and need not to be expressed only as an answer to the stress (Charron, Giegé and Lorber, 2003). They aggregate in wine to form a visible haze unless removed prior to bottling (Ferreira et al., 2001; Waters, Wallace and Williams, 1991). Thaumatococin-like protein structure was defined in grape by Marangon et al. (2014) and a physico-chemical parameters relevant for the haze formation mechanism were determined by these authors.

Beside the relevance in winemaking, thaumatococin is defined as a minor grape allergen together with the chitinases (Vassilopoulou et al., 2007). Its relevance is important, because of 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 clinically manifested by severe symptoms mainly in patients suffering of 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, no specific information exist in the literature about the grape variety based differences on the genomic or transcriptomic level for the allergens and their genes. In this study, expression of thaumatococin-like allergen was analysed in the grapes of selected varieties.

Scientific hypothesis

Thaumatococin-like protein is variety dependant in its gene expression in the red grape varieties.

MATERIAL AND METHODOLOGY

Biological material

Grapes of four red varieties of *Vitis vinifera*, L. were obtained in the season 2017 in the Sabo winery that belongs to the Malokarpatská wine region (all of the grapes were inspected to be without any infection marks). Fresh matured grapes of varieties Alibernet (syn. Odesskij čornyj), Cabernet Sauvignon (syn. Vidure Sauvignon, Petit Bouschet, Bouchet-Sauvignon, Burdeos Tintowere), Frankovka modrá (syn. Černé starosvětské, Černý muškateľ, Čierny Zierfandler, Karmazín, Lampart, Limberger, Noir de France, Blaufränkisch, Limberger, Limberger schwarz, Schwarze Fränkische, Franconia nera)

and Dornfelder were harvested and transported immediately to the laboratory where were kept them in -50 °C until further processing.

RNA extraction and cDNA synthesis

Total RNA was extracted using the GeneJet Plant RNA Purification Mini Kit (ThermoFisher) following the manufacturer instruction with a minor modification of the weight of homogenized tissue used – a 80 mg was used. RNA concentration and A260/A280 nm ratios were determined by Implen Nanophotometer. 1% agarose gels were prepared to visualize the integrity of the RNA. cDNA synthesis was performed from 72 ng of total RNA using the Tetro cDNA synthesis kit (BIOLINE) with the oligodT primer.

Thaumatococin expression analysis

A two-step protocol was used for thaumatococin expression analysis where the actin (GenBank accession AY847627) was used as the internal control during qPCR and the amplification was performed using 5x Hot FirePol@ EvaGreen@ qPCR Mix (Solis BioDyne) on a Biorad CFX qPCR thermal cycler. Following program was used: 95 °C for 10 minutes followed by 35 cycles of 95 °C for 10 seconds, 59 °C for 20 seconds and 72 °C for 20 seconds ended by dissociation curves analysis of amplified thaumatococin products by heating the amplicon from 65 °C to the 95 °C. Thaumatococin specific primers were designed on the basis of coding region of the genomic sequence stored in the GeneBank under accession AF227324.

Statistic analysis

A standard qPCR approach with the technical triplicates was used in the study and the relative expression values were calculated by the method described by Livak and Schmittgen (2001). Expression levels were determined as the number of amplification cycles obtained in the reaching of the threshold in the exponential phase of the PCR. The calculations of data presented as means and standard errors as well as graphs were prepared and performed in Microsoft Excel for Windows.

A parametric two-tailed t-test was performed for obtained thaumatococin Ct values (Smyth, 2004) using the online platform T-Test Calculator at the significance level 0.05 (Social Science Statistics, 2019).

RESULTS AND DISCUSSION

Here, expression profiles of thaumatococin-like protein were analysed in four red grape varieties – Alibernet, Cabernet Sauvignon, Frankovka modrá and Dornfelder. Dissociation curves of qRT-PCR amplified thaumatococin products calculated during the melting procedure (from 65 °C to 95 °C) showed a single melting peak with melting temperature (Tm) of 88.5 °C, indicating specific product (Figure 1). Agarose gel electrophoresis of these products confirmed amplification of a single product and no primer-dimer formation were generated during the reactions (data not shown). Control reactions of NTC generate clearly differentiated products.

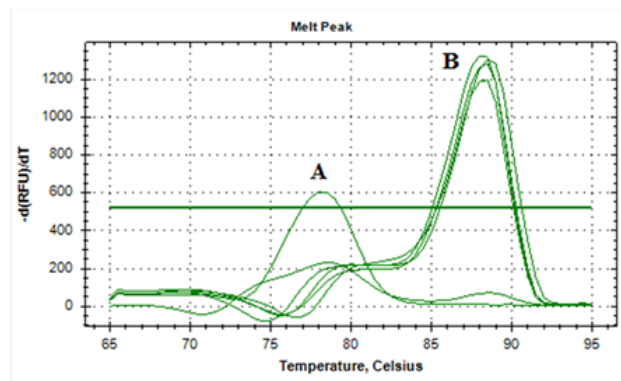


Figure 1 Melting temperatures of analyzed thaumatin products (B) and primer dimers (A) as visualized by dissociation curves.

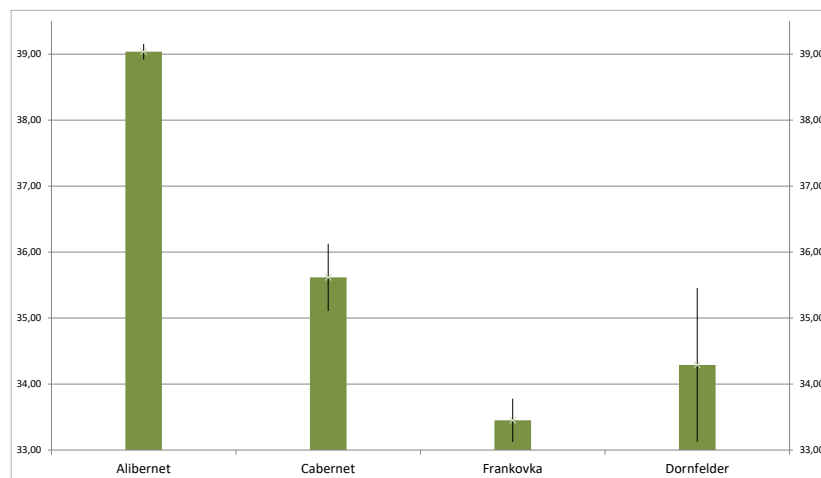


Figure 2 Characteristics of generated Cts for the triplicates of analysed red grape varieties.

For the correction of sample-to-sample variation, normalization against the actin gene was used and the generated Cts has ranged from 33.01 up to the 37.11 for the individual analysed grape varieties. Generated Cts for the thaumatin ranged from 33.1 up to the 39.11 with the variety average and standart deviations illustrated in the Figure 2.

Expression changes of thaumatin was calculated by delta delta Ct method (Livak and Schmittgen, 2001). Dornfelder was found as to have the lowest activity in thaumatin-like gene activity, counted in 100 fold percentage change, mainly when comparing to the Cabernet Sauvignon and Frankovka modrá. Alibernet, on the other side, has the expression level of thaumatin very similar when comparing to the Cabernet Sauvignon and Frankovka modrá (Figure 3).

T-test was used for generated Cts to inspect the variety dependance in generated thaumatin amplicons level in the red grape varieties (Table 1) with the results that correspond to the quantified fold changes in the thaumatin expression for the analysed grape varieties.

Food allergies, defined as an immune response to food proteins, affects approximately 8% of young children and 2% of adults in western countries and their prevalence appears to be rising like all allergic diseases (Cianferoni and Spergel, 2009). Few allergic reactions to grapes are described in literature as case reports and are usually

limited to respiratory tract mucosa (e.g. seasonal rhinoconjunctivities, asthma symptoms; anaphylactic reactions and oral allergy syndrome) (Senna et al., 2001; Brito et al., 2008; Arora et al., 2016).

Other molecular studies reported a possible relationship between allergic reactions to grapes and other botanically unrelated fruits including peach and cherry (Giannoccaro et al., 1998; Pastorelo et al., 2003). The ultimate cause of these human disorders are antifungal food allergens.

Grape allergy was reported to be specific to a certain grape variety for individual patients while tolerated others variety, or some patients may be allergic to grape but not wine (Giannoccaro et al., 1998; Bircher, Bigliardi and Yilmaz, 1999).

Different allergens were identified in grapes, one of which is thaumatin. A total of thirtythree thaumatin-like genes were identified in the grape genome (Yan et al., 2017). They are distributed in the chromosomes 1; 2; 3; 4; 6; 8; 12; 13; 14; 15; 16; 17 and 18 and varied from 573 bp up to the 24 345 bp in the length. Only a limited information exist about the natural genomic and transcriptomic variability of the thaumatin in grape genetic resources. Direct PCR was applied previously do detect the genomic sequences of thaumatin in the biological material such grapes, stormy wines and musts (Žiarovská et al., 2018).

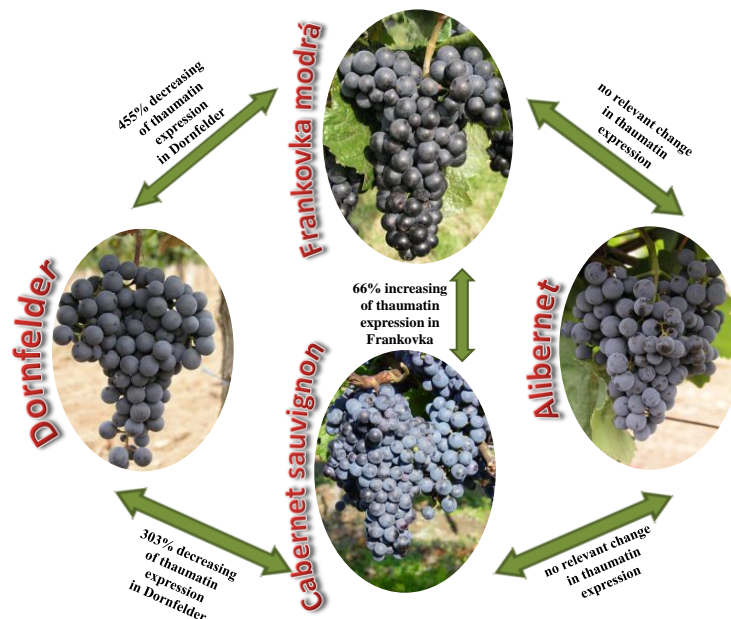


Figure 3 Expression profiles of thaumatin-like gene in the cross-comparison among analysed grape varieties.

Table 1 T-test values of thaumatin generated Cts of analysed grape varieties.

compared varieties	p-value	t-value	significant difference
A – C	0.420	-0.8	no
A – F	0.286	-1.13	no
A – D	0.002	7.02	yes
C – F	0.001	6.19	yes
C – D	0.002	6.05	yes
F – D	0.002	7.00	yes

Note: A – Alibernet; C – Cabernet; F – Frankovka; D – Dornfelder.

Expression profiles of different thaumatin-like proteins were analysed by Yan et al. (2017) in varieties Red Globe, Shang-24, Hunan-1 and Shuangyou under the inoculation of three different pathogens with the conclusion, that expression of this gene family is broadly influenced by *Botrytis cinerea*, *Elsinoe necator* and *Elsinoe ampelina*. Up to date, no specific information can be found for the expression differences among different grape varieties.

Members of the thaumatin-like protein family are described as important allergens in peaches, too (Palacín et al., 2010) and they were described to be allergens in other fruit: cherry, apple, banana, kiwi, or olive and in pollens, too. This family is supposed to belonging to panallergens that are responsible for pollen-fruit cross-reactivity (Breiteneder, 2004).

Expression of thaumatin in grape is reported up to now to be affected by different pathogens. Subsequences of inoculation by anthracnose, powdery mildew and Botrytis were 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. The other knowledge is, that PR proteins are expressed constitutively even in healthy grape fruits (Charron, Giegé and Lorber, 2003) and that is why analysing the natural varietal differences is relevant.

CONCLUSION

Thaumatin is a minor allergen in grape, but belonging to the suspected panallergens relevant to the food cross-allergy induction, its importance is quite high. Another importance of this protein is a technological one, as reported to aggregate in wine to form a visible haze unless removed prior to bottling. Expression differences of thaumatin-like gene were analysed in four red grape varieties – Alibernet, Cabernet Sauvignon, Frankovka modrá and Dornfelder. Real-time PCR approach and normalization against actin was used. None fold change in thaumatin expression was found for comparing varieties Alibernet-Cabernet and Alibernet-Frankovka modrá. Dornfelder was found to have lowest level of thaumatin expression with the expression fold change of 4.5.

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