UNIVERSITY OF PÉCS

Doctoral School of Biology and Sportbiology

The impact of TiO_2 nanoparticles treatment, developmental, and environmental factors on phenolic content and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves

Ph.D. Thesis

Sakina Bouderias

Supervisors:

Dr. László Kőrösi

Senior Research Fellow

Dr. Gábor Jakab

Professor

PÉCS, 2022

UNIVERSITY OF PÉCS

Doctoral School of Biology and Sportbiology

The impact of TiO_2 nanoparticles treatment, developmental, and environmental factors on phenolic content and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves

Ph.D. Thesis

Sakina Bouderias

Supervisors:

Dr. László Kőrösi

Senior Research Fellow

Dr. Gábor Jakab

Professor

PÉCS, 2022

1. Introduction

Over the last decades, researchers used several elicitors to increase secondary metabolite synthesis (D'Amelia *et al*., 2018; Anjum *et al*., 2019). Several studies are now using a variety of nanoparticles (NPs) as unique and effective secondary metabolite elicitors *in planta* of several plant species. The most frequent "nano-elicitors" include carbon nanotubes (CNT), silver (Ag), gold (Au), copper (Cu), zinc oxide (ZnO), and titanium dioxide nanoparticles (TiO_2 NPs) (Anjum *et al*., 2019; Khan *et al*., 2021; Lala, 2021). TiO_2 is one of the most popular commercially available nano-size materials that has found application in a variety of fields due to its wide availability, biocompatibility, low cost, non-toxicity, and high chemical stability. In nature, TiO_2 exists in four polymorphs: anatase, rutile, brookite, and TiO_2 (B). The physical and chemical features of TiO_2 depend on the crystal phase, size, and shape of the particles. For example, different phases of crystalline TiO_2 have varied band gaps, such as rutile and anatase TiO_2, which have 3.0 eV and 3.2 eV, respectively, and these band gaps impact TiO_2's photocatalytic activity. The activation of TiO_2 NPs by photon energy equal to or more than the TiO_2 band gap energy drives an electron from the valence band to the conduction band, leaving a hole in the valence band; electron-hole pairs (the charge carriers) are formed. The electron and hole participate in redox reactions with species adsorbed on the surface of TiO_2 such as H_2O and O_2 to generate reactive oxygen species (ROS). ROS are efficient against pathogens and the degradation of hazardous organic compounds (Kőrösi *et al*., 2019b). Furthermore, they play a crucial role as signal molecules for upregulating antioxidant defense in plants.

During metabolic activities such as respiration and photosynthesis, plants continually create ROS in chloroplasts, mitochondria, peroxisomes, and other cell locations. In addition, environmental variables, both biotic (e.g., fungus, viruses, bacteria, insects, and herbivores) and abiotic (e.g., high light, heat, cold, drought, salt, and nutrient deficiencies), impact plant growth and ROS generation (Sharma *et al*., 2012). At low levels, ROS operate as signaling molecules involved in growth, development, and defense. On the other hand, an overabundance of ROS in plants under stress conditions results in damaged cell membranes, deoxyribonucleic acid (DNA), protein, and other cell components, impeding plant development. Plants primarily use antioxidants to scavenge ROS (Zhao *et al*., 2020). Polyphenols have high antioxidant activity, which may help with ROS elimination (Das and Roychoudhury, 2014; Harb *et al*., 2015; Zhang *et al*., 2017).

Flavonols are polyphenols that belong to the flavonoid family. They are primarily accumulated in the epidermal cells of plant tissues in response to solar radiation (Juvany *et al*., 2013; Winkel-Shirley, 2002) to filter UV-B light while allowing photosynthetically active visible light to pass through (Liu *et al*., 2015; Gregan *et al*., 2012). Besides their photoprotective roles (Agati and Tattini, 2010), flavonols have an antioxidant function during plant response to different environmental stresses (Winkel-Shirley, 2002). Grapevine (*Vitis vinifera* L.) varieties are rich in polyphenols, especially flavonol derivatives such as quercetin and kaempferol glycosides (Kőrösi *et al*., 2019a; Teszlák *et al*., 2018; Topalović *et al*., 2012; Hmamouchi *et al*., 1996).

2. Aims of the thesis

Phenolic compounds are produced primarily in plants for their growth, development, and protection. However, its accumulation in plants is affected by many environmental and developmental factors.

The main aims of this study are to investigate the effects of TiO_2 NPs on phenolic content and antioxidant capacity in the leaves of five grapevine varieties. In addition, the effects of leaf position (age) and season on flavonol glycosides (FLGs) distribution in Cabernet Sauvignon leaves are presented.

To address these points, we studied the following:

1. Impact of TiO_2 NPs treatment on phenolic compounds in the leaves of five grapevine varieties

1. 1. To what extent can the TiO_2 NPs treatment affect the polyphenol levels and antioxidant capacity in the leaves of different grapevine varieties?

1. 2. Is there a relationship between phenolic compounds and antioxidant capacities, and can this relation be affected by TiO_2 NPs treatment?

1. 3. Can phenolic profiles and antioxidant capabilities be utilized as chemotaxonomic characteristics to distinguish grapevine varieties?

2. The age and season dependent changes in the distribution of flavonol glucoside in Cabernet Sauvignon leaves.

2. 1. How are FLGs distributed depend on the leaf position?

2. 2. Does the FLGs content of the leaves depend on the season?

2. 3. Is there a relationship between different FLGs, and can this relation be affected by seasons?

3. Materials and Methods

3. 1. Treated the leaves by TiO_2 (Degussa P25 TiO_2 NPs)

Twenty-three-year-old vines of *Vitis vinifera* L. varieties Cabernet Franc, Cabernet Sauvignon, Kadarka, Kékfrankos, and Merlot were investigated under non-irrigated conditions. Mature and healthy sun-adapted leaves from the 3^{rd}–5^{th} nodes were used for the measurements. TiO_2 NPs treatment was performed in May 2017 using 1 mg ml^{-1} Degussa P25 TiO_2 dispersion in high purity deionized water without any additives. The leaves were treated by using simple manual sprayer. Some milliliters of dispersion were sprayed onto the adaxial surface of leaves until the dispersion covered them homogenously and then allowed to dry. Control leaves were treated solely with deionized water. After two weeks of treatment, five control and five treated leaves per vine stock were collected from each variety. Three individual vines were chosen for the treatments. The collected grapevine leaves were dried at 35 °C in dark for 24 hours and then grounded in a porcelain mortar.

3. 2. Collection of leaves (leaf position)

Thirteen-year-old vines of *Vitis vinifera* L. variety 'Cabernet Sauvignon' was investigated under non-irrigated open-field conditions on the south-facing slopes of Mecsek Hills. Leaf samples were collected from randomly chosen shoots of nine individual vines in June and September 2018. Three shoots of different vines were combines and leaves were pooled at the same leaf positions. Since the developmental stage of shoots was strongly different in the two seasons, the shoots possessed 28 and 42 leaf levels (one leaf per node) in June and September, respectively. Based on our phenological monitoring of the variety Cabernet sauvignon during the vegetation period, we set up the correlation system between leaf levels and corresponding leaf age according to BBCH scale (Lorenz *et al*., 1995; Coombe, 1995; Duchene *et al*., 2010). The collected grapevine leaves were immediately frozen in liquid nitrogen, then transported to the laboratory and stored at - 25°C. The leaves were grounded in liquid nitrogen using a porcelain mortar then they were lyophilized.

3. 3. Sample preparation and extraction

25 mg of each powder sample was extracted with 1.0 ml of 60% (v/v) aqueous methanol solution acidified with formic acid (1% (v/v)), and subsequently sonicated in water bath for 30 min. The resulting suspensions were centrifuged at 20,660 × g and the supernatants were filtered through 0.22 μm PTFE syringe filters (Labex Ltd., Hungary). The obtained supernatants were analyzed by using HPLC and different assays for the determination of total phenolics and antioxidant capacity.

3. 4. High-performance liquid chromatography analysis (HPLC-DAD)

Chromatographic analysis was performed on a PerkinElmer Series 200 HPLC system using a Phenomenex Kinetex® 2.6 μm XB-C18 100 Å, 100 × 4.6 mm column. Column temperature was maintained at 25 °C. Mobile phase was composed of (A) 0.1% formic acid and (B) a mixture of 0.2% formic acid and acetonitrile (1:1). The flow rate was 1 ml min^{-1}. A volume of 5 μl of methanolic extract was injected into the HPLC system and the absorbance was monitored by a diode array detector (DAD) at 330 nm (for caftaric acid) and 350 nm (for flavonols). Calibration curves for quantification were obtained by measuring analytical standards with known concentrations.

3. 5. Total phenolic content (TPC): Folin-Ciocalteu Reaction (FCR) assay

TPC was determined using FCR method (Folin and Ciocalteu, 1927). 500 μl of diluted Folin-Ciocalteu reagent (in distilled water 1:10) was mixed with 20 μl of 10x diluted leaf extracts in cuvette. After 5 min of incubation, 500 μl of Na_2CO_3 (6% w/v) solution was added to each mixture. After 90 min of incubation at room temperature, the absorbance was measured at 760 nm by using spectrophotometer. Calibration curve was made with gallic acid, and total phenolics of leaf samples were expressed as μmol gallic acid equivalent per mg dry weight (DW).

3. 6. Ferric-reducing antioxidant power (FRAP)

FRAP provide the Fe^{3+} reducing capacity of the leaf extracts measuring the absorbance change of ferrous TPTZ complex (Szőllősi and Szőllősi Istvánné Varga, 2002). FRAP reagent was prepared by mixing 12.5 ml of acetate buffer (300 mM, pH 3.6), 1.25 ml of TPTZ solution (10 mM TPTZ in distilled water) and 1.25 ml of 20 mM $FeCl_3$ solution (in 40 mM HCl). 10 μl of

diluted leaf extracts were added to 190 µl of FRAP reagent in microplate wells. After 30 min incubation at room temperature, absorbance was measured at 620 nm in plate reader. Calibration curve was made with ascorbic acid, and FRAP data of leaf samples were expressed as µmol ascorbic acid equivalent per mg DW.

3. 7. Trolox equivalent antioxidant capacity (TEAC)

TEAC measurements were based on the method reported by Re *et al*. (1999) adapted for plant materials as described earlier (Majer and Hideg, 2012). The main reaction is the reduction of $ABTS^{\bullet+}$, which was prepared by mixing 9.7 ml of phosphate buffer (50 mM, pH 6.0), 100 µl of ABTS (0.1 mM), 100 µl of horse radish peroxidase (0.0125 mM) and 100 µl of H_2O_2 (1 mM). After 15 min incubation at room temperatures, 10µl of leaf sample extract was added to 190 µl of $ABTS^{\bullet+}$ solution, and then the conversion of the cation radical into colorless ABTS was followed by measuring the absorbance at 651 nm. Calibration curve was made with trolox and TEAC data of leaf samples were expressed as µmol trolox acid equivalent per mg DW.

3. 8. Statistical analysis

Statistical analyses were carried out using IBM SPSS 24.0 (IBM SPSS Inc., Chicago, IL, USA). Standard deviation and paired t-test were calculated on all data sets. Results were considered statistically significant at $P < 0.05$. Pearson correlation coefficients were calculated to determine the degree of correlations of FCR, FRAP, TEAC, caftaric acid, and main flavonols for both control and treated leaves. The greater absolute value of Pearson coefficient (r) indicates stronger correlation at 0.05 and 0.01 level of significance. Multivariate analyses were conducted by means of canonical discriminant functions to evaluate variety similarities using FCR, FRAP, TEAC, caftaric acid (CA), and flavonols of control leaves.

4. Results and discussion

4. 1. Phenolic compounds in grapevine leaves

Phenolic compounds have an important role in plant defence against several biotic and abiotic stresses by scavenging excess ROS. The main phenolic compounds in the methanolic leaf extract of grapevine were identified using HPLC-DAD analysis. The results showed that grapevine leaves are rich in CA and flavonols. CA and six FLGs; quercetin-3-*O*-rutinoside (Q-rut), quercetin-

3-*O*-galactoside (Q-gal), quercetin-3-*O*-glucoside (Q-glc), quercetin-3-*O*-glucuronide (Q-glr), kaempferol-3-*O*-glucoside (K-glc), and kaempferol-3-*O*-glucuronide (K-glr) were identified. CA and Q-glr are the predominant derivatives with high concentrations, while the second most abundant flavonol glycoside was Q-glc. Q-rut, Q-gal, and kaempferol glycosides were detected in a lower concentration range (Kőrösi *et al*., 2019a; Bouderias *et al*., 2020).

The high concentration of CA and Q-glr indicates their importance in grapevine leaves. These compounds may have an important role in leaf defense against UV damage. Several studies agree that these two compounds are produced in high concentrations in the plant due to their UV-protective benefits (Bouderias *et al*., 2020; Kőrösi *et al*., 2019a).

4. 2. Impacts of TiO_2 NPs on caftaric acid (CA) and flavonol glycosides (FLGs)

Hydroxycinnamic acids and flavonols are known as the most stress sensitive phenolic compounds in grapevine leaves. They are strong antioxidants, and therefore they have the ability to mitigate the adverse effects of stress-induced ROS (Latouche *et al*., 2012; Csepregi *et al*., 2016). ROS in plants behaves like double-edged sword; it is beneficial at low concentrations, but damaging at higher concentrations in the cell. TiO_2 NPs have shown that are able to produce stress, generating excess ROS in the presence of UV light. $OH^{\bullet}$, $^1O_2$, and H_2O_2 are revealed by electron paramagnetic resonance (EPR) measurements (Kőrösi *et al*., 2019a). Therefore, ROS have already been shown to act as signal molecules, triggering antioxidative defense against a multitude of abiotic stresses (Mittler, 2017). In this case, accurate dosage of TiO_2 NPs-derived ROS may have action as upregulators of the plant's own antioxidant defense. In order to study the response of the non-enzymatic defense system, we measured the CA and FLGs of grapevine leaves treated with TiO_2 NPs.

CA significantly influenced by TiO_2 NPs treatment. Its level was found high in all the treated leaves with the exception of Kékfrankos variety, which did not change after treatment. In addition, TiO_2 NPs also boosted the biosynthesis of FLGs, with the notable exception of Q-glr. This dominant flavonol decreased in treated leaves, particularly in Kékfrankos. Q-glr and CA have been demonstrated to possess high antioxidant capacities; they act as first defense line of plant leaves (Csepregi *et al*., 2016). In contrast, Esca infection increase Q-glr by 35% (Goufo *et al*., 2019).

4. 3. Effects of TiO_2 NPs on the total phenolic contents (TPC) and antioxidant capacities (AOC)

According to the results for TPC and AOC, noticeable difference among investigated grapevine leaf samples is observed. The highest TPC was identified in Kékfrankos leaf extract. This sample also showed the most effective FRAP and TEAC radical scavenger activity. The lowest values for TPC, FRAP, and TEAC were obtained in the sample of Cabernet Sauvignon and Kadarka. Interestingly that after TiO_2 NPs treatment, the general level of phenolic compounds, together with FRAP and TEAC values, were significantly influenced. All grapevine varieties enhanced the level of TPC and AOC, whereas Kékfrankos decreased significantly.

In the case of Kékfrankos, we found that the level of TPC and AOC were lower compared to control leaves. This suggests that Kékfrankos variety employed more antioxidant molecules to non-enzymatically neutralize ROS, and/or the plants could not elevate sufficiently its original antioxidant level. Consequently, decreased TEAC and FRAP values in treated Kékfrankos leaves can be explained by their unchanged CA levels and lower concentration of Q-glr.

The photocatalytically produced exogenous ROS increased the phenolic compounds and AOC level in leaves. These findings are in agreement with the natural stresses which also shown influencing on polyphenol concentrations and AOC. Similarly, to the Kékfrankos situation under photocatalytic stress, the long-term water deficient condition resulted in the depletion of TPC in grapevine leaves and roots (Król *et al*., 2014). Cold stress also resulted in a decrease of the polyphenol levels leading to a lower radical scavenging capacity in the grapevine leaves (Król *et al*., 2015).

4. 4. The relationship between phenolic compounds and antioxidant capacity, and how TiO_2 NPs treatment affects it

Also in this work, the relationship between individual FLGs, CA, TPC, and AOC values was investigated. Correlation matrixes were produced using Pearson correlation coefficients for control and treated leaves. Correlation analysis on the data set of grapevine leaves revealed that there are significant and positive correlations between individual FLGs with each other and also with CA. In addition, a significant correlation was found between FCR, TEAC, FRAP, and FLGs. Both CA and Q-glr are considered the main phenolic compounds in the leaf extracts. Strong positive correlations were found between these compounds and FCR, TEAC, and FRAP. The high antioxidant activity of these compounds may explain their presence in high concentration in the

leaves. The TiO_2 NPs treatment did not change the correlations between CA, FLGs, FCR, FRAP, and TEAC. This indicates that TiO_2 NPs have the same effect on all phenolic compounds (CA and FLGs), TPC and AOC, which led to the stability of the correlations between them after treatment.

4. 5. The five grapevine varieties' characteristics

For the characterization of the five varieties, discriminant analyses were performed using CA, FLGs, FCR, FRAP, and TEAC data sets. Phenolic profile of Cabernet Franc and Kadarka was close to each other while other varieties showed significant differences. At the same time, Cabernet Sauvignon and Kadarka, with significantly different genotypes, belonged to the same group based on their FCR, FRAP, and TEAC values. Kékfrankos, with its high AOC, was highly different from the other varieties. Different elemental composition was observed for Cabernet Sauvignon and Cabernet Franc varieties even if their genotype is very similar, because Cabernet Sauvignon is a hybrid grape, originally formed by the crossing of Cabernet Franc (a red grape) and Sauvignon blanc (a white grape) (Bowers and Meredith, 1997). Our results to the five varieties showed different foliar characteristics and seems to be distinct from each other. Previous studies also showed great agreement with our findings. Where, it turns out that the difference in chemical composition is a convenient way to distinguish between grape leaves of diverse origins (Banjanin *et al*., 2020; Gülcü *et al*., 2020; Pantelic *et al*., 2017). Additionally, it is expected that phenolic compounds such as flavonols and anthocyanins can be used as chemotaxonomies to define grapevine varieties (Mattivi *et al*., 2006).

4. 6. Impact the leaf position (age) on the distribution of FLGs

Each leaf has a different level of exposure to the environmental conditions which may be can effect on the distribution of phytochemicals and AOC in *Vitis vinifera*. The aims of this study were to compare individual FLGs of Cabernet Sauvignon leaves at different stages of maturity based on its position in a shoot. Leaves at different positions from base to apex in a shoot represented gradient decrease in the leaf age (from old to young).

The results show that the concentrations of Q-rut and Q-glr are present in high concentrations in the young and old grapevine leaves. However, the concentrations of Q-gal, Q-glc, K-glc, and K-glr are constantly decreasing towards the shoot tip, resulting that younger leaves contained lower amount of them (Bouderias *et al*., 2020). This difference in the concentrations of

FLGs may be due to their distribution and accumulation in two different leaf sides (adaxial and abaxial). Therefore, we suggest that Q-glr and Q-rut accumulate faster in the adaxial as a first line of defense, due to exposure of this part of the leaf to UV (sun). The high level of these compounds prove their strong AOC in the defense processes of grapevine from the early stage of leaf development, while the abaxial side of the leaf that was not directly exposed to the sun, accumulates Q-glc, Q-gal, K-glc, and K-glr more slowly than the first side as a second line of defense. Increase the concentration of Q-gal, Q-glc, K-glc, and K-glr in the course of time suggests that their biosynthesis was continuous during the leaf aging, allowing their accumulations. Bidel *et al*. (2015) showed that at low UV-B irradiance induced the accumulation of flavonols in the exposed epidermis without any parallel increase in the mesophyll or opposite lamina surface. Under higher UV-B irradiances, the epidermis that was not directly exposed to UV-B also began to accumulate flavonols, albeit in lower amounts than the exposed epidermis. But with multiple and successive daily exposures, flavonols accumulate progressively throughout all leaf tissues.

Indeed, in September, Q-glr level was significantly lower in older leaves. Its decreasing in last developmental stage (old leaf) may can be explained by over stress in this period of leaf life cycle. As it is known, oxidative stress plays a key role at both ends of the leaf life cycle. Maintaining on an adequate cellular ROS/antioxidant (redox) balance that allows growth and prevents oxidative damage in young emerging leaves, while later on photo-oxidative stress induces cell death in senescing leaves (Juvany *et al*., 2013). For example, The UV-B-induced Q-glr in *C. asiatica*, suggests that flavonoid-mediated UV-B protection may be conferred both by UV-B screening and the quenching of ROS. At the highest UV-B irradiance, mesophyll tissue preferentially accumulated Q-glr, and in a smaller amounts K-glr (Agati and Tattini, 2010; Agati *et al*. 2012; Bidel *et al*., 2015). In addition, in our previous study, it was showed that a strong photocatalytic oxidative stress can induce the decreasing of Q-glr content in the leaves (Kőrösi *et al*., 2019a).

The study of Bhakta and Ganjewala (2009) revealed that leaf positions (age) influence on the type and level of secondary metabolites and thereby their antioxidant properties. For instance, principal component analysis illustrated distinct differences in overall phenolic profiles between old and young tea leaves. After maturation, flavanols and phenolic acids decreased, whereas flavonols increased (Liu *et al*., 2020). i.e. flavanols and phenolic acids are more active or special

accumulate in young and mature leaves, whereas, flavonols are more efficient in old leaves. This may be good explain for our study why all individual FLGs are high in old leaves.

4. 7. Influence the seasons on the FLGs content

Leaf development, from early leaf growth to senescence, is tightly controlled by plant development and the environment (Juvany *et al*., 2013; Rankenberg *et al*., 2021). Besides checking how FLGs are affected by leaf age, the effect of seasons was also revealed. FLGs also affected by the sampling date, Q-gal, Q-glc, and K-glc were present in higher amounts in leaves collected in September. By comparing the leaf compositions in different seasons (June and September), the individual FLGs showed concentration changes with similar patterns along the shoots up to 22th leaf level in both seasons. The similarity of the concentration changes in the two seasons were remarkable for all FLGs. Even though the amount of Q-gal and Q-glc was multiplied in September compared to June, the trends in their concentration changes towards the shoot tip were remained similar (Bouderias *et al*., 2020).

The similarity of the concentration trend of FLGs from 5 to 22th node, in both seasons, indicates that initial flavonol levels in younger leaves in June will be determinant for the final flavonol contents in September. The environmental variables be key factors that determine the final amounts of flavonols in leaves. Duplied Q-glc, Q-gal and increased K-glc concentrations in September. Suggest that, besides their protective functions, likely these flavonols relate to other roles. Mierziak *et al*. (2014) showed that flavonoids may be responsible for mediating ROS-induced signaling cascades vital to cell growth and differentiation. Flavonoids can regulate auxin efflux to recognize the extracellular environment. Control of auxin transport by flavonoids can be important in the stress-induced morphogenetic response of plants.

The level of total flavonols (TF) was proved to be higher in September independently from the leaf position. Although the season influenced the TF level, there were no significant differences between the TF content of the young and old leaves in one season. UV-B radiation is one of the most effective inducers for the biosynthesis of flavonols (Kolb and Pfündel, 2005). However, the increased synthesis of flavonols in September, with the reduced daily integrated UV radiation, indicates that besides UV light, there should be another regulatory factor in the biosynthesis of FLGs.

4. 8. The relationship between flavonol glycosides and how seasons affect them

Our study described the correlations between the individual FLGs in grapevine leaves. Strong positive correlations were found between the Q-glr and Q-rut levels, while Q-glc content positively correlated with Q-gal, and K-glc contents (Bouderias *et al*., 2020).

The strongest correlation was found between Q-glc and Q-gal in both June and September. Likely this strong correlation due to similarity of glycon in two compounds. Both glycose and galactose are isomer and they have very similar structure. Therefore, they have similar or same function in plant. As it has been shown that the molecular structures can influence on the antioxidant capacity of phenolics (Cao *et al*., 2019; Ono *et al*., 2010).

5. New scientific achievements

The main aims of this study were to investigate the effects of TiO_2 NPs treatment, leaf position (age), and seasons on phenolic content and antioxidant capacity in the grapevine leaves.

Based on our results, we conclude that:

1. TiO_2 NPs treatment boosted the production of phenolic compounds in grapevine leaves.
2. TiO_2 NPs treatment increased leaf TPC and AOC of the varieties investigated (Cabernet Franc, Cabernet Sauvignon, Kadarka, and Merlot), with the exception of Kékfrankos.
3. Significant and positive correlations are found between individual FLGs with each other and also with CA and AOC. The TiO_2 NPs treatment did not change this correlation between phenolic compounds and AOC either.
4. Based on chemotaxonomic, the five grapevine varieties showed different characteristics.
5. Depending on the leaf position (age), FLGs in grapevine leaves can be divided into two groups:

5. 1. The first group: includes Q-glc, Q-gal, K-glc, and K-glr. The concentrations of these flavonols were influenced by leaf age.

5. 2. The second group: includes Q-glr and Q-rut. The concentrations of these flavonols were not influenced by leaf age.

6. In grapevine leaves level of FLGs were higher in September than that of in June.

7. Positive correlations were found between FLGs, while the strongest positive correlation was found between Q-glc and Q-gal in both June and September.

6. References

Agati G., Tattini M. Multiple functional roles of favonoids in photoprotection. *New Phytol.* (2010). 186, 786–793.

Agati G., Azzarello E., Pollastri S., Tattini M. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Sci.* (2012). 196, 67–76.

Anjum S., Anjum I., Hano C., Kousar S. Advances in nanomaterials as novel elicitors of pharmacologically active plant specialized metabolites: Current status and future outlooks. *RSC Adv*. (2019). 9, 40404.

Banjanin T., Uslu N., Vasic Z. R., Özcan M. M. Effect of grape varieties on bioactive properties, phenolic composition and mineral contents of different grape-vine leaves. *J. Food Process. Preserv*. (2020).

Bhakta D., Ganjewala D. Effect of leaf positions on total phenolics, flavonoids and proanthocyanidins content and antioxidant activities in *Lantana Camara* (L). *J. Sci. Res*. (2009). 1, 363−369

Bidel L. P. R., Chomicki G., Bonini F., Mondolot L., Soule J., Coumans M., Fisca P. L., Baissac Y., Petit V., Loiseau A., Cerovic Z. G., Gould K. S., Jay-Allemand C. Dynamics of flavonol accumulation in leaf tissues under different UV-B regimes in *Centella asiatica* (*Apiaceae*). *Planta*. (2015).

Bouderias S., Teszlák P., Jakab G., Kőrösi L. Age- and season-dependent pattern of flavonol glycosides in Cabernet Sauvignon grapevine leaves. *Sci. Rep*. (2020). 10, 14241.

Bowers J. E., Meredith C. P. The parentage of a classic wine grape, Cabernet Sauvignon. *Nat. Genet*. (1997). 16, 84–87.

Cao Y., Fang S., Fu X., Shang X., Yang W. Seasonal variation in phenolic compounds and antioxidant activity in leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja. *Forests*. (2019). 10, 624**.**

Csepregi K., Neugart S., Schreiner M., Hideg É. Comparative evaluation of total antioxidant capacities of plant polyphenols. *Molecules*. (2016). 21, 208.

Coombe B. G. Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res*. (1995). 1, 104–110.

D'Amelia V., Aversano R., Chiaiese P., Carputo D. The antioxidant properties of plant flavonoids: Their exploitation by molecular plant breeding. *Phytochem. Rev.* (2018). 17, 611–625.

Das K., Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* (2014). 2, 1–13.

Duchene E., Huard F., Dumas V., Schneider C., Merdinoglu D. The challenge of adapting grapevine varieties to climate change. *Clim. Res*. (2010). 41, 193–204.

Folin O., Ciocalteu V. On tyrosine and tryptophane determinations proteins. *J. Biol. Chem*. (1927). 73, 627–650.

Goufo P., Marques C.A., Cortez I. Exhibition of local but not systemic induced phenolic defenses in *Vitis vinifera* L. affected by brown wood streaking, grapevine leaf stripe, and apoplexy (*Esca* complex). *Plants*. (2019). 8, 412.

Gregan S. M. Wargent J. J., Liu L., Shinkle J., Hofmann R., Winefield C., Trought M., Jordan B. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon blanc grapes. *Aust. J. Grape Wine R*. (2012). 18, 227–238.

Gülcü M., Ghafoor K., Al-Juhaimi F., Özcan M. M., Uslu N., Babiker E. E., Ahmed I. A. M., Azmi I. U. Effect of grape (*Vitis vinifera* L.) varieties and harvest periods on bioactive compounds, antioxidant activity, phenolic composition, mineral contents, and fatty acid compositions of *Vitis* leave and oils. *J. Food Process. Preserv*. (2020). 00, e14890.

Harb J., Alseekh S., Tohge T., Fernie A. R. Profling of primary metabolites and flavonols in leaves of two table grape varieties collected from semiarid and temperate regions. *Phytochemistry.* (2015). 117, 444–455.

Hmamouchi M., Es-safi N., Lahrichi M., Fruchier A., Essassi E. M. Flavones and flavonols in leaves of some Moroccan *Vitis vinifera* cultivars. *Am. J. Enol. Vitic*. (1996). 47, 2.

Juvany M., Müller M., Munné-Bosch S. Photo-oxidative stress in emerging and senescing leaves: a mirror image? *J. Exp. Bot*. (2013). 64, 3087–3098.

Khan A. K., Kousar S., Tungmunnithum D., Hano C., Abbasi B. H., Anjum S. Nano-elicitation: As an effective and emerging strategy for *in vitro* production of industrially important flavonoids. *Appl. Sci.* (2021). 11, 1694.

Kolb C. A., Pfündel E. E. Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. *Plant Cell Environ*. (2005). 25, 580–590.

Kőrösi L., Bouderias S., Csepregi K., Bognár Balázs., Teszlák P., Scarpellini A., Castelli A., Hideg É., Jakab G. Nanostructured TiO_2-induced photocatalytic stress enhances the antioxidant

capacity and phenolic content in the leaves of *Vitis vinifera* on a genotype dependent manner. *J. Photochem. Photobiol. B.* (2019a). 190, 137–145.

Kőrösi L., Bognár B., Bouderias S., Castelli A., Scarpellini A., Pasquale Lea., Prato Mirko. Highly-efficient photocatalytic generation of superoxide radicals by phasepure rutile TiO_2 nanoparticles for azo dye removal. *Appl. Surf. Sci*. (2019b). 493, 719–728.

Król A., Amarowicz R., Weidner S. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta Physiol. Plant*. (2014). 36, 1491–1499.

Król A., Amarowicz R., Weidner S. The effects of cold stress on the phenolic compounds and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves. *J. Plant Physiol*. (2015). 189, 97–104.

Latouche G., Bellow S., Poutaraud A., Meyer S., Cerovic Z.G. Influence of constitutive phenolic compounds on the response of grapevine (*Vitis vinifera* L.) leaves to infection by *Plasmopara viticola*. *Planta.* (2012).

Liu L., Gregan S., Winefeld C., Jordan B. From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon blanc grape berry. *Plant Cell Environ*. (2015). 38, 905–919.

Liu Z., Bruins M. E., Bruijn W. J. C., Vincken J. P. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. *J. Food Compost. Anal*. (2020). 86, 103385;

Lorenz D. H., Eichhorn K. W., Bleiholder H., Klose R., Meier U., Weber E. Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)—codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.* (1995). 1, 100–103.

Majer P., Hideg É. Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. *Plant Physiol. Biochem.* (2012). 50, 15–23.

Mattivi F., Guzzon R., Vrhovsek U., Stefanini M., Velasco R. Metabolite profiling of grape: Flavonols and anthocyanins. *J. Agric. Food Chem*. (2006). 54, 20, 7692–7702;

Mierziak J., Kostyn K., Kulma A. Flavonoids as important molecules of plant interactions with the environment. *Molecules.* (2014). 19, 16240–16265.

Mittler R. ROS are good. *Trends Plant Sci*. (2017). 22, 1.

Khan A. K., Kousar S.,Tungmunnithum D., Hano C., Abbasi B. H., Anjum S. Nano-elicitation: As an effective and emerging strategy for In vitro production of industrially important flavonoids. *Appl. Sci*. (2021). 11, 1694.

Lala S. Nanoparticles as elicitors and harvesters of economically important secondary metabolites in higher plants: A review. *IET Nanobiotechnol*. (2021). 15, 28–57.

Ono E., Homma Y., Horikawa M., Kunikane-Doi S., Imai H., Takahashi S., Kawai Y., Ishiguro M., Fukui Y., Nakayama T. Functional differentiation of the glycosyltransferases that contribute to the chemical diversity of bioactive flavonol glycosides in grapevines (*Vitis vinifera*). *Plant Cell*. (2010). 22, 2856–2871.

Pantelic M. M., Dabic Zagorac D. C., Ciric I. Z., Pergal M. V., Relic D. J., Todic S. R., Natic M. M. Phenolic profiles, antioxidant activity, and minerals in leaves of different grapevine varieties grown in Serbia. *J. Food Compos. Anal*. (2017). 62, 76–83.

Rankenberg T., Geldhof B., Veen H. V., Holsteens K., Van de Poel B., Sasidharan R. Age dependent abiotic stress resilience in plants. *Trends Plant Sci.* (2021). 26, 7.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*. (1999). 26, 1231–1237.

Sharma P., Jha A. B., Dubey R. S., Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* (2012). 26.

Szőllősi R., Szőllősi Istvánné Varga I. Total antioxidant power in some species of *Labiatae* (Adaptation of FRAP method). *Acta. Biol. Szeged*. (2002). 46, 125–127.

Teszlák P., Kocsis M., Scarpellini A., Jakab G., Kőrösi L. Foliar exposure of grapevine (*Vitis vinifera* L.) to TiO_2 nanoparticles under field conditions: Photosynthetic response and flavonol profile. *Photosynthetica*. (2018). 56, 1378–1386.

Topalović A., Mikulic-Petkovsek M., Perovic N., Trifunovic S., Knezevic M. Phenolic composition of the leaf of grapevine cv. 'Cardinal'. *Agriculture & Forestry*. (2012). 52, 5–15.

Winkel-Shirley B. Biosynthesis of favonoids and effects of stress. *Curr. Opin. Plant Biol*. (2002). 5, 218–223.

Zhang H., Wu Z., Suo Y., Wang J., Zheng L., Wang Y. Gene expression and flavonol biosynthesis are induced by Ultraviolet-B and salt stresses in *Reaumuria trigyna*. *Biol. Plant.* (2017). 61, 246–254.

Zhao L., Lu L., Wang A., Zhang H., Huang M., Wu H., Xing B., Wang Z., and Ji R. Nano-Biotechnology in Agriculture: Use of nanomaterials to promote plant growth and stress tolerance. *J. Agric. Food Chem.* (2020). 68, 1935−1947.

7. Scientific publications and conference abstracts

7. 1. Thesis publications

Kőrösi L., **Bouderias S**., Csepregi K., Bognár B., Teszlák P., Scarpellini A., Castelli A., Hideg É., Jakab G. Nanostructured TiO_2-induced photocatalytic stress enhances the antioxidant capacity and phenolic content in the leaves of *Vitis vinifera* on a genotype-dependent manner. *J. Photochem. Photobiol. B.* (2019). 190, 137−145; (**IF: 3.261)**

Bouderias S., Teszlák P., Jakab G., Kőrösi L. Age- and season-dependent pattern of flavonol glycosides in Cabernet Sauvignon grapevine leaves. *Sci. Rep*. (2020). 10, 14241; (**IF: 4.379)**

7. 2. Other publications

Kőrösi L., Bognár B., **Bouderias S**., Castelli A., Scarpellini A., Pasquale L., Prato M. Highly-efficient photocatalytic generation of superoxide radicals by phase-pure rutile TiO_2 nanoparticles for azo dye removal. *Appl. Surf. Sci*. (2019). 493, 719−728; (**IF: 6.386)**

Bouderias S., Teszlák P., Bognár B., Csepregi K., Hideg É., Jakab G., Kőrösi L. Fotoreaktív nanorészecskék hatása vörösborszőlő-fajták levelének polifenol összetételére és tápelemtartalmára. *Kertgazdaság* (1998). 51: 3 pp. 31−42, 12 p. (2019).

Kőrösi L., Teszlák P., Bouderias S. Nanorészecskék a szőlészeti kutatásokban. *Agrofórum Extra*. 86 pp. 50-54., 5 p. (2020).

Kőrösi L., Molnár Sz., Teszlák P., Bouderias S. Magyar nemesítésű festőszőlők szín-és aromavilágának kromatográfás tanulmányozása. *Agrofórum Extra*. 91 pp. 26−31. (2021).

7. 3. Thesis conference abstracts

Bouderias S., Teszlák P., Jakab G., Kőrösi L. Nanostructured titanium dioxide as an antimicrobial agent on grapevine (*Vitis vinifera* L.) leaves: A phytotoxicological study. In: Tamás, László; Zelenyánszki, Helga (eds.) Fiatal Biotechnológusok Országos Konferenciája "FIBOK 2018": Abstract Book. Budapest, Hungary: JATEPress Kiadó, (2018). pp. 113−114., 1 p.

Bouderias S., Teszlák P., Balázs B., Csepregi K., Hideg É., Jakab G., Kőrösi L. Boosting the antioxidant capacity, phenolic content, and macro- and microelements in *Vitis vinifera* leaves by photoreactive TiO_2 nanoparticles. 8th interdisciplinary doctoral conference book of abstracts. Pécs, (2019). P. 13.

Bouderias S., Teszlák P., Czégény G., Jakab G., Kőrösi L. Application of anatase and rutile titanium dioxide nanoparticles in vineyard: response of enzymatic and non-enzymatic defense system of grapevines. 4th national conference of young biotechnologists. "FIBOK 2020": Abstract Book. Debrecen, Hungary, (2020). pp. 38., 1 p.

7. 4. Other conference abstracts

Bouderias S., Teszlák P., Jakab G., Kőrösi L. Drought stress-induced changes of flavonol profile in grapevine leaves (*Vitis vinifera* L.) In: David, Edwards; Rodomiro, Ortiz - 3rd Agriculture and Climate Change Conference. (2019). p. 1.

Teszlák P., **Bouderias S**., Jakab G., Kőrösi L. Szárazság stressz indukálta változások a szőlőlevél polifenolos összetételében in: Puskás, János (eds.) 11. Szőlő és klíma konferencia: Program és az előadások összefoglalói. (2019). p. 29.