NKFI K-112309

Multi-aspect (total and ROS specific) analysis of flavonoid antioxidant capacities for the exploitation of UV light as post-harvest manipulation of table grape biofortification

Final Project Report

INTRODUCTION & OVERVIEW

Our research was inspired by preceding publications on the post-harvest manipulation of plant metabolites in a variety of fruits. Within these, only a small number of works included grapevine, as this plant is characterized with non-climacteric ripening. This motivation from published literature met the long-standing interest of our laboratory in the effects of UV radiation as eustress and investigating the role of phenolic secondary metabolites. Our results on plant-UV interactions positioned the laboratory into the core of European UV research community. This brought the opportunity to coordinate a large scale grapevine experiment which confirmed solar UV-B as the main correlator of developmental and metabolic UV-responses (Castagna et al. 2017).

Work within the K-112309 project started with a successful proof of concept: Berries of table grapes responded to post-harvest UV radiation. Studies of berry skin extracts were complemented with *in vitro* experiments, which were aimed at the understanding of structural reasons behind the diverse antioxidant capacities of various phenolic compounds. Working with berries also highlighted the importance the connection between the enzymatic and non-enzymatic antioxidant systems: flavonoids linking these two aspects of defence acting as direct antioxidants as well as substrates to phenolic peroxidases. Exploring the role of hydrogen peroxide and peroxidases in UV-responses thus became an integral part of the project. During the last phase of the project, a new aspect came to our attention: the possibility of a dual role of B6-vitamers as direct antioxidants and peroxidase effectors, which was explored in model experiments (Czégény et al. 2019). This new result needs and deserves further attention and a new project proposal is in preparation to this end.

SUMMARY OF RESULTS

(1) Berry skins respond to post-harvest UV irradiation

Four cultivars, two with black and two with white berries were studied; and various energies of UV irradiation (315-400 nm UV-A and 280-315 nm UV-B) were applied. We identified two possible effects. We have shown that UV irradiation of ’Black Sultanina’ berries resulted in a decrease in phenolic contents, which was due to a selective, marked decrease in flavonol (quercetin-3-O-glucoside and quercetin-3-O-glucuronide) contents; while phenolic acid content was unaffected. The decrease which was observed 2-24 hours after UV-treatment recovered during 48 h storage at room temperature. We made two further, important observations. First, in a comparative study of the effect of UV-A and UV-B irradiation, we found that the decrease in quercetin derivatives was ***not a direct UV photo-effect***. Second, using peroxidise staining, we detected a marked increase in peroxidase enzyme activities after the UV irradiation (in response to both UV-B and UV-A) showing that the observed decrease is ***due to the oxidation of quercetins as peroxidase substrates*** (Csepregi et al. 2019).

A comparative study of 'White Sultanina' and 'Queen of Vineyards' berries showed the possibility of a positive effect and demonstrated that responses depend on the UV transparency of berry skins, i.e. the depth of UV penetration. In addition to the temporary negative effect observed in the thinner skin cultivar 'White Sultanina', similarly to what was found in the black berries of 'Black Sultanina', ***an increase in flavonoid content*** was found in the thicker-skinned 'Queen of Vineyards'. The source of metabolic energy for this latter effect is suggested to be derived from ***increased berry skin photosynthesis*** (Csepregi et al., under review).

It is to be noted that some of the studied grapevine varieties were affected by biotic factors and the choice of disease free berry clusters in consecutive years was challenging, hence results obtained with the second black variety 'Muscat Hamburg' lacked reproducibility and were not suitable for publication.

(2) Structure-function aspects of total and ROS-neutralizing antioxidant capacities of phenolic compounds

In vitro characterization of phenolic compounds targeted both general (total) antioxidant capacities and specific ROS neutralization. This work was carried out using 37 test compounds representing various flavonoids and phenolic acids.

First we carried out a comparative analysis of compounds using, the Trolox Equivalent Antioxidant Capacity (TEAC), the Ferric Reducing Antioxidant Potential (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, in addition to the Folin-Ciocalteu reagent reactivity (FCR). We found that antioxidant hierarchies depended on the choice of assay and applied ANOVA analyses to explore underlying structure-TAC dependencies. In addition to statistically confirming the empirically established connection between flavonoid ring-B catechol and high TEAC or FRAP, new correlations were also found. In flavonoids, (i) ***hydroxyl groups on ring-B had a positive effect*** on all four TAC assays; (ii) the presence of a ***3-hydroxyl group on ring- C increased TEAC and FRAP***, but had no effect on DPPH or FCR; (iii) Phenolic acids lacking a 3-hydroxyl group had significantly lower FRAP or DPPH than compounds having this structure, while TEAC or FCR were not affected (Csepregi et al. 2016). As a confirmation *in planta*, antioxidant capacities and flavonoid contents (especially quercetin derivatives) were found to be strongly correlated in berry skins (Csepregi et al. 2019), as well as in leaves of grapevine (Csepregi et al. 2016) and of a model plant (Csepregi et al. 2017).

In a second *in vitro* study, we explored the structural background of hydrogen peroxide and singlet oxygen neutralizing capacities in comparison with UV absorbing properties. We found that singlet oxygen and hydrogen peroxide detoxifying capacities of flavonoids were positively correlated, largely due to the ***strong positive effect of the hydroxylation of the C-ring in position-3***. In accord, 3-O-***glycosylation decreased the ROS-reactivities*** of quercetin and myricetin but eradicated the hydrogen peroxide reactivity of kaemferol. ***B-ring polyhydroxylation increased the hydrogen peroxide antioxidant function but decreased UV-B absorption***. UV-A (315–400 nm) absorption was On the other hand, the ***B-ring C2-C3 double bond increased UV-A absorption***, either in itself or in combination with the C4 oxo-group (Csepregi & Hideg 2018).

The above results achieved with test compounds served as valuable discussion points when evaluating berry skin responses to UV radiation.

(3) Hydrogen peroxide housekeeping as the key factor of UV-responses

The importance of UV-induced peroxidase upregulation has been shown to affect berry skin UV responses (Csepregi et al. 2019). This aspect, the effect of UV on the balance between H_2O_2 production and neutralization was explored further in a leaf model, because these samples had higher uniformity and higher base levels of the enzyme than berries. Studies required ***technical advancement in H_2O_2 detection and in measuring non-enzymatic H_2O_2 neutralization*** (Mátai & Hideg 2017, Csepregi & Hideg 2016, respectively). Our results established ***a preference of peroxidase fortification over other antioxidant enzymes*** in acclimation to UV (Czégény et al. 2016), and showed that it was ***achieved by specific UV-responsive isoforms*** (Rácz et al. 2018).

DISSEMINATION

In the framework of project K-112309, the PI and project participants authored 9 peer reviewed journal publications (cumulative impact factor 25.4) and gave 13 international conference presentations.

PUBLIC DISSEMINATION – POPULAR SCIENCE

The PI gave two invited talks at events organised in connection to the International Year of Light 2015 (one at the Pécs regional group of HAS and the second organised at national level by the Biology Division of HAS), as well as public lectures on the occasion of Fascination of Plants Day in 2016 and 2017 organized by the Department of Plant Biology at University of Pécs. Aims and results of the project were reported on these occasions. The Fascination of Plants Day events also included simple demonstrations of research methodology as well as delineating potential benefits of expected results.

The whole research team participated at Researchers' Night 2018 and 2019, gave public lectures and practical demonstration on the colourful and hidden world of flavonoids in plants.

COLLABORATION

The majority of the experiments were carried out at the PI's laboratory, with the help of senior research partners (not on project budget) from the Research Institute of Viticulture and Oenology (RIVE) and the Department of Organic and Medicinal Chemistry (DOMC) of University of Pécs. An international collaboration with the Leibniz Institute (Grossbeeren, Germany) provided us the HPLC analytical background which was lacking locally during the first phase of the project. Partners from University of Pécs contributed with discussing results, provided the berry samples (RIVE) and advised methods development (DOMC). Senior research partner Péter Teszlák from RIVE has chosen and harvested plant material and contributed to discussing results. HPLC analysis in the second half of the project was carried out at RIVE, and the PI's laboratory allocated sufficient budget to research chemicals to this purpose.

EDUCATION

Kristóf Csepregi (junior research partner -1) defended his PhD in May 2017, and his thesis was based on project results. In the framework of the project, he gave two poster and short oral presentations at international conferences and first-authored five journal publication. He was employed on project budget until 31-Aug-2017 instead of the planned 31-Dec-2017 end date, because he was offered a university contract. Nevertheless, he contributed to the work until the end of the project.

Gyula Czégény (junior research partner -2) was employed on budget for the whole duration of the project. He defended his PhD in January 2018 and his thesis included project results. He gave four well-received conference presentations, first authored one project publication, and is the senior (corresponding) author of a second paper. This latter work is based on results of an MSc student's work, which Gyula Czégény and the PI co-supervised, and which was awarded at the 2017 National Conference of Student's Scholarly Circles (OTDK).

OTHER PROJECT EVENTS

Due to shifting of the starting date (the project proposal included a start on 1st of September 1014, the proposal was accepted with 1st of January 2015 as a starting date), the project missed grapevine berry sampling possibilities in 2014. Work was re-scheduled accordingly and the first post-harvest UV irradiation experiments were carried out in September-October 2015. This delay was compensated by the prolongation of the project up to the end of September 2019.

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