CLOSING REPORT – NKFI Grant KH-129615

Project KH-129615 had two main aims: (1) to expand our laboratory infrastructure with an HPLC equipment, and (2) to utilize this it in order to increase the scientific potential of ongoing research work. Both aims have been fulfilled, although our research activities had to be adjusted, due to university restriction in response to the SARS-Cov-2 pandemic, which resulted in limited excess to laboratory facilities during several months, and strongly limited international research collaborations in 2020-21. The possibility to extend this project by 180 days has been very much helpful.

1. PURCHASE AND SET UP OF THE NEW EQUIPMENT

The HPLC system was purchased in March 2019 as the public procurement procedure required a several months after the official start of the project. There was a marked, six-month delay in laboratory renovation and refurbishing at the University of Pécs, which was financed by 20765-3/2018/FEKUTSTRAT. Consequently, the HPLC system was first set up at a temporary location, then moved to the new laboratory with the help of the seller's product engineer March 2020. While setting up at the first location was included in the price of the equipment, costs of the second moving had to be covered from project budget. This was manageable, because the HPLC system was purchased at a lower-than-expected price due the seller's discount. Moreover, we were also able to purchase a special compact stand for service units used for sample preparation. Project participant, Kristóf Csepregi has attended a special, advanced HPLC course, which was postponed several times, before it was held in compliance of government public health guidelines in October 2020. Due to his improved laboratory skills and the new infrastructure, the following research projects were completed:

2. RESULTS

2.1. ANTIOXIDANT TESTS OF PURE COMPOUNDS

We have completed a comparative structure-function analysis of phenolic compounds as antioxidants (Csepregi & Hideg, Phytochem. Anal. 2018, 29, 129-136), earlier, and this work served as the basis of the current project. The task was to utilize, and modify these techniques for the characterization of carotenoids, and to include data on new phenolic compounds into the data base. Test compounds were partly provided by partner laboratories and partly purchased from commercial sources on project budget. The current challenge was to modify existing methodology in order to evaluate both hydrophilic and hydrophobic antioxidants under the same assay conditions. This was necessary to compare antioxidant capacities of carotenoids with other antioxidants occurring in leaves, such as ascorbate or flavonoids, and was achieved using methyl-beta-cyclodextrin. Results showed diverse responses to different total antioxidant capacity tests, in accordance to previous reports. Reactivities

to 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS-TEAC) were the highest for β-carotenoid among the studied compounds, ca. 3-times higher than α-tocopherol. Xanthophylls showed high antioxidant capacities: 1.5-2-times higher than α-tocopherol, depending on their structure. These results indicated a putative new role of xanthophylls in leaves, in addition to their known function in energy dissipation under stress conditions. Realisation of such functions in vivo depends on actual conditions, plant species and age, etc. Our experiments with sun exposed grapevine leaves showed that that acclimation to higher sunlight fluxes did not depend on this function (see Section 2.2.3.). Nevertheless, it cannot be excluded that acclimative energy dissipation responses are supported by direct antioxidant function when environmental conditions are harsher and the oxidative pressure is stronger. Results of in vitro antioxidant capacity tests have been submitted for publication and are under review. These were also reported by the PI at the 3rd Network Meeting of the International UV4Plants Association for Plant UV Research (UV4Plants), which was held virtually in 2020.

2.2. LEAF METABOLITE ANALYSES

2.2.1. BARLEY

In the framework of a collaboration with the Global Change Research Institute (Brno, Czech Academy of Sciences) we studied the contribution of flavonoids to the UV tolerance of barley. This work was intended to combine the analysis of extracts from leaves harvested in Brno in stress experiments, as well as the evaluating test compounds representing major metabolite groups in Pécs (see Section 1.2). Earlier results from the Czech group showed the importance of lutonarin over saponarin in acclimation to solar UV in a multiple factor experiment. Our team complemented these findings with characterizing the antioxidant capacity of these flavonoids and other compounds identified as stress-responsive in barley leaf extracts with HPLC. A set of special, purified compounds and leaf extracts were provided by the Czech partner and were analysed using methods developed in our laboratory. Results confirmed the importance of hydroxylation of ring-B in the antioxidant function of flavonoids. This we reported earlier for the C-3 flavonols found in grapevine (Csepregi & Hideg 2018), and new results confirmed a similar role in flavones of barley. Collaboration with the Czech partners on the role of barley phenolics in acclimation to various growth light conditions (+/- UV-A or UV-B) was slowed down by the SARS-Cov-2 pandemic, because the planned personal transfer of samples on liquid nitrogen has not been possible due to travel restrictions. In order to make up for the delay in this topic, which was beyond our means to influence, a similar project has been successfully completed with a partner in Germany, using three Brassicaceae vegetables as detailed below.

2.2.2. BRASSICACEAE

In the framework of a collaboration with the Georg-August-University Göttingen and the Leibniz Institute of Vegetable and Ornamental Crops, we examined how growth light conditions affected carotenoid, phenolic acid and flavonoid glycoside profiles in three leaf vegetables: kale, kohlrabi and rocket salad. Plants were grown indoors, in the green-house or in growth chambers and standard photosynthetically active radiation (PAR) was supplemented with daily doses of UV-B alone or in combination with additional green or blue light. HPLC metabolic profiles were compared to those of plants grown under PAR only. Growth light conditions had no significant effects on carotenoid profiles, and the major groups of responsive metabolites were identified as flavonol glycosides: either acylated quercetin and kaempferol glycosides (kale and kohlrabi) or quercetin glycosides only (rocket salad). The main factor affecting metabolite profiles was the UV-B, and its effect was stabilized by blue light treatment following UV irradiation. The less pronounced change in carotenoids may be explained by the applied relatively low PAR (100-400 µmol photons m^{-2} s^{-1}) keeping conditions under the range of photoinhibition. This hypothesis was supported by identifying minor changes only in ROS specific antioxidant capacities of leaf extracts. These changes in singlet oxygen or hydroxyl radical neutralizing capacities were, however, correlated with changes in contents of specific compound groups, especially in rocket salad, indicating the importance of these compounds in acclimation.
Results were published as a joint paper in 2021, in Frontiers in Plant Science, an open access, D1 ranked journal. In addition to scientific novelty, results of this project have the potential of applications in horticulture. Plans for further joint research are under discussion with partners in Germany. We plan to apply for an NN-type international cooperation NKFI project with a German grant holder laboratory in 2022, if there will be such call for proposals.

2.2.3. GRAPEVINE

Sun exposed and canopy covered grapevine leaves collected in two consecutive years were analysed for antioxidant and metabolite contents. The latter involved HPLC analyses of carotenoids and phenolic compounds. These analyses were partly performed with the newly purchased instrument in Pécs and partly in collaboration with the Centre for Agricultural Research Plant Physiology Department (Martonvásár, Hungary). This study, complementing work in the framework of another NKFI project, is aimed at following leaf responses to a sudden change in their light conditions. When part of the grapevine canopy is removed, shade leaves become exposed to high intensity PAR and UV radiation. The working hypothesis assumed an equal contribution of carotenoids and special metabolites in acclimation to new conditions. Photosynthesis (gas exchange) measurements showed that leaves acclimated successfully to new light conditions, and their water use efficiency was up to the level of values characteristic to sun leaves within two days. Such change from shade to full sun conditions

induced a change in metabolite profiles, and these changes support defence against oxidative stress. A distinct function of different compound groups was established: The main result of the work is that carotenoids prevent oxidative stress rather as energy dissipating metabolites than antioxidants, and that the antioxidant function is fulfilled by flavonoids. Data collection and all analyses are complete, and manuscript discussions among future co-authors are in progress. The work is going to be submitted to a Q1 level journal.

The HPLC system purchased from project budget has also been essential in understanding changes in grapevine berry metabolites brought about by UV radiation of berry clusters in the laboratory. The working hypothesis of this study was that berry skin photosynthesis is a source of defence active compounds. The idea of this research originated in a recently closed NKFI project, in which we studied postharvest metabolite UV responses of mature berries. The importance of berry skin photosynthesis has been characterized in developing green berries, but it is less recognized in mature berries. To provide data on this latter developmental stage, we combined berry skin photosynthesis measurements with the analysis of antioxidant capacities and metabolite profiles. Results showed that mature grapevine berries have photosynthetically active tissues capable of dynamic changes even several hours postharvest, and suggested that changes in photochemistry may contribute to postharvest metabolic responses of berry skins. Results also supported the potential of postharvest manipulation of fruit qualities with UV irradiation.
The work was published in 2021 in Photosynthetica, an open access Q1 journal.

DISSEMINATION

Plant stress physiology related conferences on, which were scheduled for 2020 were postponed due to the SARS-Cov-2 pandemic, with the exception of one international conference, where the PI gave a talk on some of the yet unpublished results of the project.
Two open access works have already been published; one more manuscript is under review and a fourth one is in preparation.

Csepregi, K., Teszlák, P., Rácz, A., Czégény, Gy., Kőrösi, L., Hideg, É. (2021) Changes in grapevine berry skin photochemistry may support metabolic responses to postharvest treatment by ultraviolet light. *Photosynthetica* 59: 286-293. (Q1, IF_{2020} = 2.562)

Neugart, S., Majer, P., Schreiner, M., Hideg, É. (2021) Blue light treatment but not green light treatment after pre-exposure to UV-B stabilizes flavonoid glycoside changes and corresponding biological effects in three different Brassicaceae sprouts. *Frontiers in Plant Science* 11: 611247. (D1/Q1, IF_{2020} = 4.402)

Hideg, É. (2020) Quantifying the invisible? – Evaluating acclimative responses of leaf photosynthesis to UV in indoor and outdoor experiments. *Conference talk at the 3rd Network Meeting of the International UV4Plants Association for Plant UV Research UV4Plants held virtually 13-16th of October 2020.*

A biology BSc student was involved in the project performing antioxidant assays under the supervision of project participant Kristóf Csepregi. The student submitted and successfully defended her graduation thesis January 2021.

The HPLC system is to be involved at MSc level teaching, when regular tuition resumes. Results have already been included in an online seminar given on plant specific metabolites in the spring semester of 2021 by Kristóf Csepregi.