UNIVERSITY OF PÉCS

Doctoral School of Biology and Sportbiology

Priming of the antioxidant system affects biotic and abiotic stress responses of plants

PhD thesis

Anikó Mátai

Supervisors:

Dr. Éva Hideg
professor

Dr. Gábor Jakab
professor

PÉCS, 2019

1. Introduction

Global climate change and shortage of resources are some of the biggest challenges mankind has to overcome in the near future. Many suggest that a post-fossil or bio society is the solution when every organic resource (food, fuel, etc.) is based on plants. However, currently used agricultural methods are unable to produce enough biomass for the above-mentioned reasons; therefore a "redesign" of crop plants is needed. To achieve this, we may use modern molecular technologies or exploit the genetically encoded potential of plants. For the latter, it is crucial to understand the physiological background of plant responses to changes in the environment.

Many of these responses were shown to involve reactive oxygen species (ROS) either as initiators of cellular damage or as parts of signalling pathways evoking acclimative responses. ROS are generated during basic physiological processes of plants in chloroplasts, mitochondria, peroxisomes, plasma membrane, apoplast, cell wall and endoplasmic reticulum (Goraya and Asthir, 2016). The chemistry behind ROS production from oxygen is either energy transfer (yielding singlet oxygen, $^1O_2$) or electron transfer (resulting primarily in superoxide radical, $O_2^{\bullet-}$; then hydrogen peroxide, H_2O_2 and hydroxyl radical, $^{\bullet}OH$) (Apel and Hirt, 2004). In order to keep cellular ROS concentrations low, plants employ a network of enzymatic and non-enzymatic antioxidants (Gill and Tuteja, 2010).

Plants are usually well-adapted to their natural environment; however, unfavourable changes in the environment evoke stress responses. Natural stress factors may be biotic (e.g. virus, bacteria, fungi, insects, herbivores) or abiotic (e.g. high light, freezing, heat, drought, salt, nutrient deficiency). Anthropogenic stressors are special group of abiotic factors, which arise as consequences of human influence: such as air pollution, heavy metal pollutions, acid rain, higher solar UV doses, xenobiotics, etc.

Plants may be tolerant or sensitive to a certain environmental factor, and these responses have been studied in laboratory experiments extensively. Under stress conditions which include enhanced ROS production, upregulation of the antioxidant defence is an essential constituent of tolerance. In a natural environment, it is more likely that several factors affect plants at the same time. The simultaneous presence of two factors may be additive, lead to an effect that is equal to the sum of the effects caused by the two factors applied separately. Acclimation to one stress factor may result in increased protection against a second factor (cross-resistance) or, on the contrary, in increased sensitivity to a second factor (cross-sensitivity) (Jansen et al., 2019). Priming is a special case of induced cross-resistance, it is a unique physiological state that

enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells (Conrath et al., 2006). Priming young plants under controlled conditions in order to enhance their future stress tolerance outdoors has a substantial potential in agriculture, especially in horticultural industry (Wargent et al., 2011).

2. Aims

Acclimation to changes in environmental factors depends on the balance of prooxidants and antioxidants. In order to understand and manipulate plant stress responses both sides are to be examined. Effective priming as a long term goal may only be achieved after the dissection of natural plant stress responses. To support solving this complex problem we focused on the following topics and research questions:

1. H_2O_2 as a stress marker molecule.
   1.1. Optimisation of a rapid and low-cost spectrophotometric assay detecting H_2O_2 in leaf extracts.
   1.2. Does H_2O_2 have a role in the recovery from 'Bois noir' disease of grapevine caused by phytoplasma?

2. β-aminobutyric acid as a priming agent.
   2.1. Does β-aminobutyric acid induce recovery from virus infections of grapevine?
   2.2. Does β-aminobutyric acid affect tobacco leaf responses to UV-B radiation?

3. UV-B, a modulator of plant stress responses.
   3.1. Does UV-B induce cross-resistance against drought in tobacco leaves?
   3.2. Does UV-B affect the antioxidants of young tobacco leaves developed under drought?

3. Materials and methods

Plant growth and treatment conditions

N. benthamiana and *N. tabacum* plants were grown on standard soil in growth chambers under 175 μmol/m^2/s PAR, long day conditions (16 h/8 h light/dark, 25 °C/20 °C) and 70% relative air humidity. *N. benthamiana* plants were used in drought (10 days, achieved with water withdrawal) related experiments, *N. tabacum* plants were used in β-aminobutyric acid (BABA, single treatment with 290 μM final concentration) related experiments. The applied UV-B corresponded to 6.9 and 5.4 kJ/m^2/d biologically effective radiation as calculated using the Biological Spectral Weighting Function (Flint and Caldwell, 2003) in the drought and in the BABA related experiments, respectively.

Woody cane cuttings with two-nodal segments derived from grapevine leafroll associated virus 1 (GLRaV-1) infected *V. vinifera* L. cvs. 'Olasz rizling', 'Korai piros veltelíni' and 'Leányka' plants were planted in 300 ml plastic pods filled with hydrated-perlite in greenhouse. BABA was added as soil drench to the plants once every week for 6 weeks after the first leaves appeared. Outdoor experiments were performed using *V. vinifera* L. cv. 'Kékfrankos' plants in a private vineyard near Villány, which were diagnosed with 'Bois noir' phytoplasma infection in 2014. Photosynthetic measurements and leaf H_2O_2 concentration assay were performed in the summer and autumn of the following year.

Photosynthetic measurements and leaf pigment analysis

Photosynthetic rate (*A*, μmol CO_2/m^2/s), transpiration rate (*E*, mmol H_2O/m^2/s), stomatal conductance to H_2O (*gs*, mol H_2O/m^2/s), intercellular CO_2 concentration (*Ci*, μmol CO_2/mol) were measured using an LCA-4 type open system infrared gas analyser (IRGA) and water use efficiency (WUE) was calculated as *A*/*E* (μmol CO_2/mmol H_2O). Variable chlorophyll fluorescence parameters were assessed using the MAXI-version of the Imaging-PAM (Pulse-Amplitude-Modulation). PSII maximum quantum efficiency (F_v/F_m) and the light acclimated yield of photochemical conversion (Y(II)) were calculated, together with quantum yields of regulated (Y(NPQ)) and non-regulated (Y(NO)) non-photochemical energy quenching according to Klughammer and Schreiber (2008). Chlorophyll and flavonoid contents of leaves were estimated using the DUALEX® Scientific leaf clip equipment (Goulas et al., 2004).

Leaf hydrogen peroxide content determination

Three colorimetric assays assessing H_2O_2 concentrations were compared. The tested methods were based on the oxidation of one of the following chromophores: potassium iodide (KI), 3,3'-diaminobenzidine (DAB) or xylenol orange (XO) (Velikova et al., 2000; Šnyrychová et al., 2009; Sigma-Aldrich Technical Bulletin, 2013), each displaying a characteristic absorption change upon oxidation. For absorption characterization and H_2O_2 concentration range determination tests, a series of 1-100 nM H_2O_2 solutions were dissolved in 100 µL total volume of distilled water, 100 mM potassium-phosphate buffer (pH 7.0), 70% (v/v) ethanol or 6% (v/v) trichloroacetic acid (TCA) and 1 mL of KI, DAB or XO solution were added.

Based on a comparison of sensitivity, linearity and changes in chromophore absorption spectra (Mátai and Hideg, 2017), the XO-based assay was chosen and optimized for leaf H_2O_2 content determination. 100 mg fresh leaf discs were homogenized in 1 mL ice cold 6% TCA using pestle and mortar. Extracts were centrifuged (15,000 × *g*, 10 min, 4ºC) and 100 µL of supernatants were added to 1 mL XO. Absorption was measured at 560 nm after 30 min incubation at room temperature.

Total and specific antioxidant capacities

Total antioxidant capacities of leaf extracts were determined using two electron transfer based photometric methods. One of these, the TEAC assay is based on measuring the reduction of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) by antioxidants contained in the sample (Majer and Hideg, 2012). In the other method the samples' ferric reducing capacities are assessed by following the reduction of ferrous the TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) complex (Szőllősi and Szőllősi-Varga, 2002).

In addition to total antioxidant capacities, ROS specific non-enzymatic antioxidant capacities of leaf extracts were also assessed. The first assay is based on the observation that the oxidation of 2,5-diphenyl-3,4-benzofuran (DPBF) by photodynamically generated $^1O_2$ (methylene blue illuminated with 50 µmol/m^2/s red, 600-650 nm light) is decreased by $^1O_2$ reactive antioxidants (Majer et al. 2014). Second, in a separate assay, estimating non-enzymatic H_2O_2 neutralising was based on the photometric detection of iodine (I_2) from the reaction between H_2O_2 and potassium iodide (KI), and the ability of H_2O_2 reactive compounds to limit this reaction (Csepregi and Hideg, 2016; Mátai et al., 2019b). Third, $^{\bullet}OH$ reactivity of antioxidants contained in leaf extracts were measured based on the capacity to inhibit the radical induced oxidation of terephthalic acid (TPA, 1,4-benzenedicarboxylic acid) into fluorescent 2-hydroxyl-terephthalate (Šnyrychová and Hideg, 2007).

Activities of two enzymes were characterized in leaf samples: superoxide dismutase (SOD) activity was measured as the inhibition of formazan generation from the reaction of $O_2^{\bullet-}$ and nitro blue tetrazolium (NBT) (Song et al., 2007). Total peroxidase activity was determined using *o*-phenylenediamine (OPD) (Fornera and Walde, 2010) (*N. benthamiana*) or ABTS (Childs és Bardsley, 1975) (*N. tabacum*) as substrate.

Polymerase chain reaction (PCR)

For the detection of phytoplasma, total DNA was extracted, and a multiplex nested PCR was performed according to the guidelines of the European and Mediterranean Plant Protection Organisation (EPPO, 2007). The program for the first PCR was: 1. 92 °C 90 sec, 2. 92 °C 40 sec, 3. 55 °C 40 sec, 4. 72 °C 70 sec, 5. GO TO 2 29x, 7. END. Products were diluted 1000x. The program for the second PCR was: 1. 92 °C 90sec, 2. 92 °C 40 sec, 3. 55 °C 40 sec, 4. 72 °C 70 sec, 5. GO TO 2 34x, 6. 72 °C 120 sec, 7. END.

For the detection of GLRaV-1, total RNA was extracted and cDNA was synthesised using a primer designed for coat protein of the virus. The PCR program was: 1. 94 °C 4 min, 2. 94 °C 30 sec, 3. 53 °C 45 sec, 4. 72 °C 1 min, 5. GO TO 2 29x, 6. 72 °C 5 min, 7. END.

For the examination of plant gene expressions, total RNA was extracted, and cDNA was synthesised using an Oligo(dT)$_{18}$ primer. The selected marker genes were: GST1 (glutathione-S-transferase), HSR1 (putative hypersensitive response marker), PR1 (pathogenesis-related protein 1) and PR4 (pathogenesis-related protein 4). The PCR program for all the genes was: 1. 94 °C 4 min, 2. 94 °C 30 sec, 3. 56 °C 45 sec, 4. 72 °C 1 min, 5. GO TO 2 34x, 6. 72 °C 5 min, 7. END.

Statistical analyses

Treatment groups contained 3-5 plants as biological repetitions (n=3-5). Differences between two group means were assessed with two-sample Student's t-tests for either equal or unequal variances, depending on results of F-tests. Results were considered significant when the test gave $p<0.05$ for the null hypothesis that the two means were equal.

In two-factor experiments, two-way ANOVA was used for each variable to test three null hypotheses (first factor has no effect, second factor has no effect, there is no interaction between the factors). Rejections of null hypotheses ($p<0.05$) were verified with Tukey's *post-hoc* test.

Calculations were carried out using either the MS Excel Analysis ToolPak (Version 2007, Microsoft Corporation, Redmond, WA, USA) or the PAST software (Hammer et al. 2001).

4. Results and discussion

Optimisation of a spectrophotometric assay detecting H_2O_2 in leaf extracts.

Probably the most popular spectrophotometric assays are the ones using KI, DAB or XO. We compared these methods in three steps, in order to examine: (i) the absorption characteristics of the oxidized chromophores, (ii) the concentration range of H_2O_2 detection, (iii) whether leaf metabolites other than H_2O_2 interfered with the assay. For the last step, leaf extracts were spiked with exogenous H_2O_2 which was attempted to recover using the assays. All measurements were repeated using four different solvents: distilled water, potassium-phosphate buffer, ethanol or TCA.

Our results show that among the tested conditions extraction into and assaying in 6% TCA or 70% ethanol with XO as chromophore proved the most suitable methods to detect H_2O_2 in plant derived samples. However, it is recommended to calculate cellular concentration relying on a series of H_2O_2 spiked samples rather than a calibration line acquired in the corresponding solvent only (Mátai and Hideg, 2017). This optimised method was used on grapevine leaves infected with 'Bois noir' phytoplasma.

Recovery from 'Bois noir' disease does not depend on hydrogen peroxide accumulation

In 2014, *Vitis vinifera* L. cv. 'Kékfrankos' plants in a private vineyard near Villány, Hungary showed characteristic symptoms of phytoplasma diseases. '*Candidatus* Phytoplasma solani' infection was verified using nested PCR. Plants were retested for phytoplasma infection the following year in July and in September. Furthermore, gas exchange performance, photochemical processes and leaf H_2O_2 content assessing measurements were performed in July. Surprisingly, some of the examined plants recovered since they were infected in 2014, but the pathogen was not detected from leaf samples collected in 2015.

In the case of most phytoplasma diseases the key element of recovery is H_2O_2 accumulation which serves as a signal to induce defence responses (Gambino et al., 2013; Musetti et al., 2005). However, in 'Kékfrankos' grapevines, H_2O_2 levels in recovered plants were the same as in the healthy ones, and it was higher in the infected ones. These results

suggest that against '*Candidatus* Phytoplasma solani' hydrogen peroxide is rather a typical biotic stress signal and not the key molecule in recovery (Mátai et al., under review).

β-aminobutyric acid may protect plants against grapevine viruses

There are some diseases from which plants are unable to recover in a natural way. Virus infections of grapevines (*Vitis vinifera* L.) belong to these diseases. However, it is possible to induce cross-resistance against the virus artificially. Woody cane cuttings with two-nodal segments from grapevine leafroll associated virus 1 (GLRaV-1) infected *V. vinifera* L. cvs. 'Olasz rizling', 'Korai piros veltelíni' and 'Leányka' plants were treated with 2,9 mM BABA once a week for 6 weeks after the appearance of the first leaves. Three leaves were analysed from the lower (L), middle (M) and upper (U) part of the shoot of each plant.

SA and JA dependent signalling pathways are important in biotic stress responses, both were characterised with the expression of a pathogenesis related (PR) protein encoding gene. *PR-1* was the marker gene for SA dependent signalling, while *PR-4* was the marker gene for JA dependent signalling. In control plants, no pathogen was detectable in U leaves if both pathways were induced. However, gene expression was slightly cultivar dependent. BABA-treated 'Olasz rizling' and 'Korai piros veltelíni' plants had similar expression patterns as corresponding untreated controls. In BABA treated 'Leányka' plants, marker genes were not expressed, although the virus titre decreased. According to these results, both SA and JA dependent signalling pathways are needed against GLRaV-1 infection, which is in contrast with the classic antagonistic model. Furthermore, we found that BABA probably has a protective role, independently from both examined signalling pathways (Csikászné Krizsics et al., 2014).

β-aminobutyric acid modifies leaf acclimation to consecutive UV-B treatment

BABA was recently recognised as an internal stress signal in plants. This compound is naturally present in plants at low concentrations but shows 5- to 10-fold increase upon exposure to abiotic or biotic stresses (Thevenet et al., 2017). This finding gave the idea to examine whether BABA affected non-enzymatic antioxidants in leaves and its potential priming effect in connection with abiotic stress responses. *N. tabacum* plants were treated with BABA once as a soil drench before exposure to a relatively low dose of UV-B radiation for 9 days. After the treatments, leaf photochemistry and pigment analysis were carried out, then leaf extracts were used to determine H_2O_2 content, UV absorbing and total antioxidant capacities, as well as non-enzymatic H_2O_2 and $^{\bullet}OH$ scavenging capacities.

Our experiments showed that both BABA and UV-B increased total non-enzymatic antioxidant capacities. The effect of BABA alone was smaller than that of UV-B. Nevertheless, positive effects of the two treatments as single factors were additive in the two-factor experiment for both antioxidant capacities. Additivity suggests that either (i) BABA and UV-B stimulate the biosynthesis of different metabolite groups with antioxidant properties or (ii) both treatments increase levels of the same metabolites but act at different levels of regulation. Distinct effects of BABA and UV-B on the flavonoid index and the total amount of extractable UV-absorbing compounds support the first assumption. However, the observed interaction between the effects of BABA and UV-B on a ROS-specific antioxidant function indicates a more complex mechanism. Single factor positive effects of BABA and UV-B were lost when the two factors were present simultaneously, and leaves which were UV-B-exposed after the BABA treatment showed the same low H_2O_2 neutralization as untreated leaves. The following model may explain our findings: Because BABA lowers leaf H_2O_2 concentrations, BABA-treated leaves need lower non-enzymatic H_2O_2 neutralization for a successful acclimation to UV-B. Consequently, BABA-treated samples are less prone to oxidative damage by H_2O_2-derived hydroxyl radicals, and this condition allows lower cellular levels of $^{\bullet}OH$ -neutralising compounds. This interaction of BABA with acclimative UV-B responses appears different from those between BABA and other abiotic stress factors (Mátai et al., 2019a).

UV-B induces cross-resistance against drought in mature leaves

Our earlier studies showed that plant responses to low UV-B doses were acclimatory including increased antioxidant enzyme activities and higher amounts of non-enzymatic antioxidants. These are also the characteristics of acclimative responses to several other stressors, suggesting the potential of UV-B to enhance survival under biotic or abiotic stress conditions. We examined antioxidant responses of tobacco (*N. benthamiana*) plants to low, acclimative UV-B doses and drought achieved by limited watering. The parallel two-factor treatment served as a simulation of the natural multifactorial environment, while the sequential two-factor treatment (UV-B followed by drought) was performed to analyse whether a UV-B pre-treatment modified concomitant responses to drought. This study was part of an ongoing series of experiments in our Department focusing on priming stress tolerance in crop plants with low UV-B doses. In the work described in the PhD thesis, we examined enzymatic and non-enzymatic ROS specific antioxidant responses to single factor drought, single factor UV-B, as well as in parallel or sequential two-factor treatments.

All studied antioxidant parameters increased in response to at least one of the treatments. Several components of the leaves' antioxidant defence gave positive responses to one treatment only, either to UV-B (SOD activity and $^{\bullet}OH$ scavenging) or to drought (non-enzymatic H_2O_2 scavenging) when applied as single factors, and all these effects remained significant in the two-factor treatment. Five among the seven assayed parameters (peroxidase enzyme activities, flavonoid index, TEAC, FRAP, and $^1O_2$ neutralizing) increased in response to both drought and UV-B as single treatments. When UV-B and drought were applied together, peroxidase activity was only UV-B and not drought driven. On the other hand, both drought and UV-B remained significant positive factors for the other four parameters. Moreover, positive effects of drought and UV-B on TEAC and FRAP were additive and synergistic on flavonoid index. An interesting new aspect of the present work is that UV-B as pre-treatment had a long-term positive effect on leaf flavonoid index and antioxidant capacity assessed as FRAP, detectable even 10 days after the irradiation. When UV-B pre-treatment was followed by drought in the sequential two-factor treatment, UV-B was maintained as significant positive factor. Moreover, effects of UV-B pre-treatment and drought were additive on both FRAP and flavonoid index, although not synergistic as observed in the parallel treatment. The present study demonstrates the capability of UV-B pre-treatment to augment drought inducible increase in leaf antioxidant capacity and flavonoid index (Mátai et al., 2019b).

Younger leaves are less sensitive to drought

Developmental age has been shown to affect antioxidant capacities and stress responses. Developing, young leaves (YL) of the same *N. benthamiana* plants whose mature leaf (ML) responses were described in the previous chapter were analysed. In untreated *N. benthamiana* plants, YL had lower weight and chlorophyll content than ML. In the absence of stress factors, there were no significant differences between flavonoid indexes of YL and ML. YL had ca. 10% lower maximum PSII quantum efficiency than ML but showed no significant differences in either light acclimated leaf photochemistry or non-photochemical quenching. YL had lower total antioxidant capacities, peroxidase enzyme activity, and contained less $^1O_2$ or $^{\bullet}OH$ scavenging compounds than ML. On the other hand, YL had higher SOD and non-enzymatic H_2O_2 scavenging than ML.

Drought in itself decreased ML and YL fresh weights. UV-B alone had no effect on the fresh weight of YL, which emerged during the 10 days of this treatment, indicating a network complex, a whole plant level UV-B response. YL antioxidants did not respond to drought as single treatment or in combination with UV-B. Peroxidase or SOD enzyme activities were not

responsive to UV-B either. Positive antioxidant responses of YL to UV-B as single factor included TEAC and FRAP, as well as $^{\bullet}OH$ and $^{1}O_2$ scavenging, and these were maintained as positive main effects in the parallel drought and UV-B treatment. On the other hand, the parallel application of drought and UV-B had a synergistic effect on flavonoid index of YL. This result confirms the central role of flavonoids in stress tolerance. In conclusion, younger leaves are naturally less sensitive to drought and UV-B has an indirect effect through the increase of non-enzymatic antioxidants (Mátai et al., 2019b).

5. Summary

Plants have developed a complex system of enzymatic and non-enzymatic antioxidants in order to adapt to an ever-changing environment and to minimise damage by oxidative stress. Plant response to a potential stressor depends on the equilibrium of antioxidants and pro-oxidants. Environmental factors as potential stressors may activate similar antioxidant responses. Consequently, these factors may influence each other's effect on the plants' defence system. This cross-talk could be exploited in order to prime antioxidant responses protecting crop plants against biotic and abiotic stress without the use of ecologically dangerous chemicals. Based on our results, we conclude that:

1. H_2O_2 as a stress marker molecule.
   1.1. We optimised a rapid and low-cost xylenol orange based spectrophotometric assay to assess H_2O_2 concentration in leaf extracts.
   1.2. We found that H_2O_2 is rather a biotic stress marker and does not have a role in the recovery of *V. vinifera* cv. 'Kékfrankos' from 'Bois noir' disease caused by *'Candidatus* Phytoplasma solani' phytoplasma.

2. β-aminobutyric acid as a priming agent.
   2.1. We started to optimise a β-aminobutyric acid treatment which may induce recovery of grapevine from GLRaV-1 infection.
   2.2. We found that a single-dose β-aminobutyric acid treatment improved tobacco leaf acclimation to a consecutive UV-B treatment.

3. UV-B, the modulator of plant stress responses.

3.1. Our results show that UV-B pre-treatment has a long-lasting effect on mature leaves' antioxidant system leading to cross-resistance against drought in tobacco.

3.2. We observed that younger leaves are naturally less sensitive to drought and only direct UV-B radiation affects the antioxidant system of young leaves in tobacco.

6. References

Apel, K., Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **55**, 373–399.

Childs, R.E., Bardsley, W.G. (1975) The steady-state kinetics of peroxidase with 2,2'-azinodi-(3-ethylbenzthiazoline-6-sulphonic acid) as chromogen. *Biochem. J.* **145**, 93–103.

Conrath, U., Beckers, G.J.M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., Mauch-Mani, B. (2006) Priming: getting ready for battle. *Mol. Plant Microbe Interact.* **19**, 1062–1071.

Csepregi, K., Hideg, É. (2016) A novel procedure to assess the non-enzymatic hydrogen-peroxide antioxidant capacity of metabolites with high UV absorption. *Acta Biol. Hung.* **67**, 447–450.

Csepregi, K., Hideg, É. (2018) Phenolic compound diversity explored in the context of photo-oxidative stress protection. *Phytochem. Anal.* **29**, 129–136.

EPPO (2007) Diagnostic. Grapevine flavescence dorée phytoplasma. *Bulletin OEPP/EPPO Bulletin* **37**, 536–542.

Flint, S.D., Caldwell, M.M. (2003) A biological spectral weighting function for ozone depletion research with higher plants. *Physiol. Plant* **117**, 137–144.

Fornera, S., Walde, P. (2010) Spectrophotometric quantification of horseradish peroxidase with *o*-phenylenediamine. *Anal. Biochem.* **407**, 293–295.

Gambino, G., Boccacci, P., Margaria, P., Palmano, S., Gribaudo, I. (2013) Hydrogen peroxide accumulation and transcriptional changes in grapevines recovered from Flavescence dorée disease. *Phytopathol.* **103**, 776–784.

Gill, S.S., Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**, 909–930.

Goulas, Y., Cerovic, Z.G., Cartelat, A., Moya, I. (2004) Dualex: a new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. *Appl. Opt.* **43**, 4488–4496.

Goraya, G.K., Asthir, B. (2016) Magnificant role of intracellular reactive oxygen species production and its scavenging encompasses downstream processes. *J. Plant Biol.* **59**, 215–222.

Jansen, M.A.K., Bilger, W., Hideg, É., Strid, Å., Aphalo, P., Brelsford, C., Klem, K., Mátai, A., Llorens, L., Nezval, J., Nybakken, L., Ryan, L., Sharma, A., Schenke, D., Solhaug, K.A., Spunda, V., Verdaguer, D., Yan, Y., Urban, O. (2019) Editorial: Interactive effects of UV-B radiation in a complex environment. *Plant Physiol. Biochem.* **134**, 1–8.

Klughammer, C., Schreiber, U. (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Appl. Notes* **1**, 27–35.

Majer, P., Hideg, É. (2012) 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.* **50**, 15–23.

Majer, P., Neugart, S., Krumbein, A., Schreiner, M., Hideg, É., (2014) Singlet oxygen scavenging by leaf flavonoids contributes to sunlight acclimation in *Tilia platyphyllos*. *Environ. Exp. Bot.* **100**, 1–9.

Mátai, A., Hideg, É. (2017) A comparison of colorimetric assays detecting hydrogen peroxide in leaf extracts. *Anal. Methods* **9**, 2357–2360.

Mátai, A., Jakab, G., Hideg, É. (2019a) Single dose β-aminobutyric acid irrigation modifies tobacco (*Nicotiana tabacum* L.) leaf acclimation to consecutive UV-B treatment. *Photoch. Photobio. Sci.* **18**, 359–366.

Mátai, A., Nagy, D., Hideg, É. (2019b) UV-B strenghtens antioxidant responses to drought in *Nicotiana benthamiana* leaves not only as supplementary irradiation but also as pre-treatment. *Plant Physiol. Biochem.* **134**, 9–19.

Mátai, A., Teszlák, P., Jakab, G. Recovery of *Vitis vinifera* cv. 'Kékfrankos' from 'Bois noir' disease does not depend on hydrogen peroxide accumulation. *Elbírálás alatt*

Musetti, R., Sanità di Toppi, L., Martini, M., Ferrini, F., Loschi, A., Favali, M.A., Osler, R. (2005) Hydrogen peroxide localisation and antioxidant status in the recovery of apricot plants from European stone fruit yellows. *Eur. J. Plant Pathol.* **112**, 53–61.

Sigma-Aldrich Tecnical Bulletin (2013) https://www.sigmaaldrich.com/content/dam/ sigma-aldrich/docs/Sigma/Bulletin/pd1bul.pdf.

Šnyrychová, I., Ayaydin, F., Hideg, É. (2009) Detecting hydrogen peroxide in leaves in vivo - a comparison of methods. *Physiol. Plant.* **135**, 1–18.

Šnyrychová, I., Hideg, É. (2007) The first application of terephthalate fluorescence for highly selective detection of hydroxyl radicals in thylakoid membranes. *Funct. Plant Biol.* **34**, 1105–1111.

Song, N.H., Yin, X.L., Chen, G.F., Yang, H. (2007) Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. *Chemosphere* **68**, 1779–1787.

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

Thevenet, D., Pastor, V., Baccelli, I, Balmer, A., Vallat, A., Neier, R., Glauser, G., Mauch-Mani, B. (2017) The priming molecule β-aminobutyric acid is naturally present in plants and is induced by stress. *New Phytol*. **213**, 552–559.

Velikova, V., Yordanov, I., Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* **151**, 59–66.

Wargent, J.J., Elfadly, E.M., Moore, J.P., Paul, N.D. (2011) Increased exposure to UV-B radiation during early development leads to enhanced photoprotection and improved long-term performance in *Lactuca sativa*. *Plant Cell Environ.* **34**, 1401–1413.

7. List of scientific publications

Thesis publications

Mátai, A., Jakab, G., Hideg, É. (2019) Single dose β-aminobutyric acid irrigation modifies tobacco (*Nicotiana tabacum* L.) leaf acclimation to consecutive UV-B treatment. *Photoch. Photobio. Sci.* **18**, 359–366. **Q2, IF: 2,902**

Jansen, M.A.K., Bilger, W., Hideg, É., Strid, Å., Aphalo, P., Brelsford, C., Klem, K., **Mátai, A.**, Llorens, L., Nezval, J., Nybakken, L., Ryan, L., Sharma, A., Schenke, D., Solhaug, K.A., Spunda, V., Verdaguer, D., Yan, Y., Urban, O. (2019) Editorial: Interactive effects of UV-B radiation in a complex environment. *Plant Physiol. Biochem.* **134**, 1–8. **Q1, IF: 2,718**

Mátai, A., Nagy, D., Hideg, É. (2019) UV-B strenghtens antioxidant responses to drought in *Nicotiana benthamiana* leaves not only as supplementary irradiation but also as pre-treatment. *Plant Physiol. Biochem.* **134**, 9–19. **Q1, IF: 2,718**

Mátai, A., Hideg, É. (2017) A comparison of colorimetric assays detecting hydrogen peroxide in leaf extracts. *Anal. Methods* **9**, 2357–2360. **Q1, IF: 2,073**

Czégény, Gy., **Mátai, A.**, Hideg, É. (2016) UV-B effects on leaves – Oxidative stress and acclimation in controlled environments. *Plant Sci.* **248**, 57–63. **D1, IF: 3,437**

Csikászné Krizsics A., **Mátai A.**, Nagy Á., Kovács S., Végh B.É., Werner J., Jakab G. (2014) Indukált rezisztencia alkalmazása a szőlő szürkerothadás és vírusfertőzés okozta kártétele mérséklésére. *G. Agric.* **19**, 73–77. **IF: –**

Thesis publication currently under review

Mátai, A., Teszlák, P., Jakab, G. Recovery of *Vitis vinifera* cv. 'Kékfrankos' from 'Bois noir' disease does not depend on hydrogen peroxide accumulation. *Under review*

Thesis conference abstracts

Mátai, A., Nagy, D., Hideg, É.: A tale of two factors: Can UV-B modify drought responses? In: Gaberscik, A., Germ, M., Neugart, S., Jenkins, G.I., Robson, M., Abram, D. (eds.) Abstracts of the UV4Plants International Association of Plant UV Research 2nd Network Meeting.

Ljubljana, Slovenia: Department of Biology, Biotechnical Faculty, University of Ljubljana, 2018, p. 39. **ISBN: 978-961-6822-47-3**

Mátai, A., Nagy, D., Hideg, É.: External factors modifying acclimative leaf UV-B responses. In: Christiansen, S.K., Bendevis, M. (eds.) Abstract Book for the Plant Biology Europe Conference in Copenhagen. Copenhagen, Denmark: Department of Plant and Environmental Sciences, University of Copenhagen, 2018, p 82. **ISBN: 978-87-996274-1-7**

Mátai, A., Jakab, G., Hideg, É.: Alacsony dózisú UV-B sugárzásra dott válaszának módosítása küldő tényezőkkel. In: Szalka, É., Bali Papp, Á., Molnár, Z. (eds.) XXVII. Óvári Tudományos Napok 'Fenntartható Agrárium és Környezet Az Óvári Akadémia 200 Éve – Múlt, Jelen, Jövő' Összefoglalói. Mosonmagyaróvár, Magyarország: VEAB Agrártudományi Szakbizottság, Széchenyi István Egyetem Mezőgazdaság- és Élelmiszertudományi Kar, 2018, p. 211. **ISBN: 978-615-5837-14-2**

Mátai, A., Nagy, D., Hideg, É.: Antioxidáns szintű válaszok Nicotiana benthamiana növényekben szárazság és UV-B sugárzás hatására. In: Györgyey, J. (ed.) A Magyar Növénybiológiai Társaság XII. Kongresszusa. Szeged, Hungary: Magyar Növénybiológiai Társaság, 2017. p. 36. **ISBN: 978-963-12-9736-2**

Mátai, A., Nagy, D., Hideg, É.: Combined effects of drought and UV in growth chamber experiments. In: Urban, O., Jansen, M.A.K. (eds.) Modulation of plant UV-responses by environmental factors. Book of Abstracts. Brno, Czech Republic: Global Change Research Institute, The Czech Academy of Sciences, 2017. p. 14. **ISBN: 978-80-87902-18-9**

Mátai, A., Teszlák, P., Hideg, É., Jakab, G.: Physiological changes in field grown grapevine leaves infected by „Bois noir" phytoplasma. In: Plant Biology Europe EPSO/FESPB 2016 Congress Abstracts. Paper ID: 248.

Mátai, A., Rácz, A., Czégény, Gy., Hideg, É.: Balancing between H2O2 generation and neutralisation during acclimation to UV-B. In: Czégény, Gy., Hideg, É. (eds.) 1st Network Meeting of UV4Plants, International Association for Plant UV Research: Abstract Book. Pécs: University of Pécs Institute of Biology, 2016. p. 52. **ISBN:978-963-429-048-3**

Mátai, A., Werner, J., Szegedi, E., Jakab, G.: BABA induced resistance against virus infection in grapevine. Aachen, Germany, 2015.09.06-2015.09.10. PR Proteins and Induced Resistance against Pathogens and Insects

Mátai, A., Czégény, Gy., Hideg, É.: Detecting hydrogen peroxide in tobacco leaves responding to light stress. Aveiro, Portugal, 2015.08.31-2015.09.04. 16th Congress of the European Society for Photobiology

Csikász-Krizsics, A., **Mátai, A.**, Nagy, Á., Kovács, S., Végh, B., Werner, J., Jakab, G.: BABA (β-aminovajsav) által indukált szürkerothadás és vírusfertőzés elleni védelem szőlőben. Szőlőtermesztési és Borászati Tudományos Konferencia 2015.

Mátai, A., Jakab, G.: Effect of beta-aminobutyric acid treatment on the spread of grapevine viruses. In: Györgyey, J. (ed.) 11th Congress of the Hungarian Society of Plant Biology: Book of Abstracts. HAS Biological Research Centre, Szeged, 2014. p. 50.

Csikász-Krizsics, A., **Mátai, A.**, Nagy, Á., Kovács, S., Végh, B., Werner, J., Jakab, G.: BABA (β-aminobutyric acid) induced resistance against grey mould and virus infection in grapevine. In: Bardin, M., Mauch-Mani, B., Mazzotta, S., Nicot, P., Pieterse, C., Poessel, J.-L., Ponchet, M., Schmitt, A. (eds.) Induced resistance in plants against insects and diseases: Leaping from success in the lab to success in the field. Darmstadt: 2013. pp. 429-432. **ISBN:978-92-9067-267-8**

Other publications

Kocsis, M., Csikász-Krizsics, A., Szata, B.É., Kovács, S., Nagy, Á., **Mátai, A.**, Jakab, G. (2018) Regulation of cluster compactness and resistance to Botrytis cinerea with β-aminobutyric acid treatment in field-grown grapevine. *Vitis* **57**, 35–40. **Q2, IF: 0,508**

Del-Castillo-Alonso, M.Á., Castagna, A., Csepregi, K., Hideg, É., Jakab, G., Jansen, M.A.K., Jug, T., Llorens, L., **Mátai, A.**, Martínez-Lüscher, J., Monforte, L., Neugart, S., Olejnickova, J., Ranieri, A., Schödl-Hummel, K., Schreiner, M., Soriano, G., Teszlák, P., Tittmann, S., Urban, O., Verdaguer, D., Zipoli, G., Martínez-Abaigar, J., Núñez-Olivera, E. (2016) Environmental Factors Correlated with the Metabolite Profile of Vitis vinifera cv. Pinot Noir Berry Skins along a European Latitudinal Gradient. *J. Agric. Food Chem.* **64**, 8722–8734. **Q1, IF: 3,154**

Hideg, É., **Mátai, A.**, Bognár, B., Kálai, T.: EPR Detection (Spin Probes) In: Santi Nonell, Cristina Flors (szerk.) Singlet Oxygen: Applications in Biosciences and Nanosciences, Volume 2. 332 p. Cambridge: Royal Society of Chemistry (RCS), 2016. pp. 121-134. **ISBN:978-1-78262-697-8**

Kuczmog, A., Galambos, A., Horváth, S., **Mátai, A.**, Kozma, P., Szegedi, E., Putnoky, P. (2012) Mapping of crown gall resistance locus Rcg1 in grapevine. *Theor. Appl. Genet.* **125**, 1565–1574. **Q1, IF: 3,658**

Scientometrics:
Cumulative impact factor: 21,168 (thesis: 13,848)
Citations: 48 (independent: 33)
h-index: 4