Report on short term missions

UV4Plants: our experience from visiting two research centers in Germany

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We have been studying how solar UV and visible radiation affect plant growth, performance, gene expression and accumulation of metabolites as part of our Ph.D. studies. To monitor the responses in natural light conditions, we performed several outdoor experiments in the experimental field at the Viikki campus of the University of Helsinki. Challenges of these experiments are changes in weather conditions, such as from clear to cloudy sky, rainy to completely dry days, and even in the summer, temperature occasionally dropping to nearly zero degrees at night or rising to thirty degrees in full sunlight. In order to create different solar light treatments, we use Plexiglas and polycarbonate filters which exclude selected wavelengths of UV and visible radiation. Plants are either germinated and grown under filters, or transferred from growth rooms to outdoors for solar light treatments. In both cases, fluctuation in environmental conditions causes high variation in the collected data, requiring ample replication within each experiment and in time to ensure reproducibility. With the high number of uncontrolled variables, it is also difficult to pinpoint one specific factor causing the variation in responses among replicates. Day length is one of the other major limitations of outdoor summer experiments in Helsinki (60° North) where days are very long during summer. *Arabidopsis thaliana* is one of our model plants which flowers much earlier under long-day conditions than under short-day conditions. Therefore, performing a complete growth cycle outdoor experiment with this species in Helsinki during summer is difficult.

Figure 8.1: View of the hall where the sun simulators are located, in a redeployed building that used to be the home of a small nuclear reactor.

In order to perform experiments in stable environmental conditions, we collaborated with Dr. Andreas Albert, a physicist, and Dr. Barbro J. Winkler, a biologist, from Prof. Jörg-Peter Schnitzler's research group at the Research Unit Environmental Simulation (EUS), Helmholtz Zentrum, Munich. This collabor-

Figure 8.2: View of the sun simulator used in the experiments at EUS.

ation gave us an opportunity to use the sun simulators at the EUS facility (Figs. 8.1 and 8.2). They are growth chambers with lighting conditions very similar to natural sunlight and yet they provide a controlled environment, enabling stable conditions for both short- and long-term experiments. During our visit, we familiarized ourselves with the EUS facility and got acquainted with other researchers working at the Helmholtz Zentrum Munich. Most of the work related to the sowing of seeds, transplantation, irrigation and setting up the solar simulator chamber was carried out by the personnel of the EUS facility under the supervision of Dr. Andreas Albert.

Our experiment was performed as a series of four replications in time (February–April 2015 and October–November 2015). The main aim of this experiment was to compare the short-term (6 h) and long-term (21 days) effects of UV and blue components

Figure 8.3: *Arabidopsis* plants from the experiments at EUS.

of simulated solar radiation on the growth, gene expression, and metabolite accumulation and composition of *Arabidopsis thaliana* plants (Fig. 8.3). We used four genotypes of Arabidopsis: wild type Landsberg *erecta*, UV-B photoreceptor (UVR8) mutant *uvr8*-2, UV-A and blue light photoreceptor (Cryptochromes 1 and 2) mutant *cry1cry2* and transparent testa 4 (*tt4*) mutant which has a mutation in the flavonoid biosynthesis pathway.

Plants were randomly distributed under five light treatments to study the effect of UV-B (wavelength 280–315 nm), UV-A (315–400 nm), blue (400–500 nm), short UV-A (315–350 nm) and long UV-A (350–400 nm). For the treatments, we used similar optical filters (glass and Plexiglas) to those used in our field experiments. The chamber consisted of two cuvette systems one for short– term treatment and another for long–term treatment. Photographs of the plants were taken at the end of each round to quantify the projected rosette area. At the end of the experiment, plants were harvested in liquid nitrogen and stored at −80°C for gene expression and metabolite accumulation analysis.

The European Plant Phenotyping Network (EPPN) funded this experiment, which gave us an opportunity to experience a different environment and work culture in a laboratory abroad. This collaboration was essential for producing high-quality research

by combining the expertise of Dr. Pedro J. Aphalo's research group at the University of Helsinki with Prof. Jörg-Peter Schnitzler's research group at the Helmholtz Zentrum.

The Helmholtz Zentrum is located in a pleasant and nature-surrounded area, away from the city noise. It has regular public transport connections to different parts of Munich, but sometimes the frequency of the transportation was lower in the evenings and weekends, which caused us some problems. In addition, working in a completely new environment required some time to adjust, to learn the location of the equipment and the way things work. However, after a few days, we integrated well into the system and managed to work more efficiently. Dr. Andreas Albert and Dr. Barbro Winkler, who made us feel welcome, gave us a lot of advice and helped us in the practical issues related to our experiment and stay in Munich.

During each round, we ground the frozen samples and transported them to the University of Helsinki. The gene expression study is in progress and being carried out at the University of Helsinki. To assess the metabolite accumulation and composition, we are collaborating with Dr. Susanne Neugart from Prof. Monika Schreiner's research group at the Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany. This collaboration was one of the many positive outcomes from our 1st Network Conference of UV4Plants held on 30–31 May 2016 in Pécs, Hungary.

In early 2017 Neha Rai travelled to Großbeeren, Germany, staying as a visiting researcher for one month at Prof. Monika Schreiner's lab. Großbeeren is a small town located on the outskirts of Berlin. Both the institute and guesthouse were located on the same campus, hence very convenient for work purposes. Prof. Monika Schreiner's research group has excellent expertise and equipment for the identification and quantification of plant secondary metabolites such as flavonoid glycosides and glucosinolates

Figure 8.4: The lab bench at Großbeeren, Germany

(Fig. 8.4). There was also an opportunity to quantify the hormone abscisic acid (ABA) under the guidance of Prof. Susanne Baldermann and PhD student David Schröter. For extracting metabolites and ABA, we used freeze dried samples and followed standard protocols. This research visit provided an opportunity to learn the principles behind HPLC-mass spectrometry and also hands-on experience with different sample extraction methods and data processing. The visit was funded by the EMBO short-term fellowship (Ref: ASTF 570-2016) granted to Neha Rai.

From our experience, we highly recommend the use of solar simulators in plant UV research and in general any plant research requiring a steady, easily adjustable environment with natural-like light conditions. We also encourage all young researchers to take advantage of available opportunities for visiting other laboratories, which allow not only training but also the development of a network of contacts and collaborators for the future.

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Neha Rai is a doctoral student in Sensory Photobiology and Ecophysiology of Plants (SenPEP) group working under Dr. Pedro J. Aphalo at University of Helsinki, Finland. She has been studying the molecular responses of plants exposed to solar and simulated UV and blue radiation. She is also assessing the molecular responses of UV- drought interaction in plants. Previously, she has worked as a research assistant at the Laboratory of Photosynthetic membranes at the Biological Research Centre, Szeged, Hungary. She holds her Master's degree in Plant Biology and Biotechnology from University of Hyderabad, India. She did her master's thesis on understanding the role of osmolytes in rescuing the damage of photosynthetic apparatus of salt induced *Chlamydomonas reinhardtii*. She did her Bachelor's degree in Botany Honors from Banaras Hindu University, India. In addition to her research activities she is keenly interested in creative writing and photography

Sari Siipola completed her Masters thesis in Plant Biology in 2011 at the University of Helsinki. In her thesis, she studied effects of UV and blue light on pea plants' secondary metabolism and growth. Currently Sari Siipola is studying towards her PhD. In her Doctoral thesis, she will discuss effects of UV and blue light on plant physiology and structure, and the possible use of these responses in plant production. In addition to Plant Biology, Sari Siipola has studied Environmental Biology and Science communication.

Yan Yan has specialized and completed a Master programme in Ecology during September 2012 to June 2015 in the school of Life Science of Lanzhou University, China, during which she focused on functional analysis of the UDP-Glucose Pyrophosphorylase gene family from *Populus euphratica* and *Populus pruinosa*. Currently, she is doing PhD studies in DPPS doctoral programme of the University of Helsinki. Her study subject are the different responses to solar blue and UV radiation of different UV and drought sensitive cultivars of the legumes species *Vicia faba* and *Medicago truncatula*: looking at connections between responses from whole plant, physiological and molecular levels. She is currently visiting the lab in Großbeeren, in relation to the analysis of samples from an experiment carried out in Helsinki.

UV4Plants Association's and UV4growth COST Action's role. This experiment was imagined and planned during UV4growth and UV4Plants meetings. It directly involves members from three different institutions, and was funded through various sources. In part by a large grant from the Academy of Finland to Pedro J. Aphalo, additional funding for use of the solar simulators from EPPN (European Plant Phenotyping Network) and for a planning visit by Andreas Albert and Barbro Winkler to Helsinki, from EMBO for N.R.'s visit to Susanne Neugart's lab in Großbeeren, from the doctoral programme in plant science (DPPS) of the university of Helsinki for other travel expenses. Not only the visits, but the whole experiment were made possible by the UV4Plants association and its predecessor, the UV4growth COST Action.