European Plant Phenotyping Network

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D16.1: Summary of access arrangements for HMGU

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Executive Summary

Objectives: The infrastructure at HMGU enables phenotyping under realistic and reproducible multi-stress conditions. In Expo- and SunSCREEN facilities, the adaptation and phenotype of plants under present and future environmental conditions can be studied in multifactorial experiments. The SignalSCREEN platform enables phenotyping of early events in plant-microbe interactions at the seedling and young plant level. We offer access to our facilities to scientific users, especially young scientists, as this increases the general level of knowledge.

Main Results: Eleven accesses were provided to nine different user groups (SignalSCREEN 5, ExpoSCREEN 4 and SunSCREEN 2). The experiments were mainly performed on crop plants, but also on the model plant species *Arabidopsis thaliana*. The plants were phenotyped for their response to cultivation conditions or their susceptibility to abiotic and biotic stresses. Despite the distance and travel constraints associated with COVID-19, we trained nine persons (4 PhD students, 3 postdocs, 2 senior scientists) from five user groups in all accesses to HMGU installations. The experiments allowed the users to obtain whole-plant (phytopathology) data for their respective experimental systems, to conduct experiments under fully controlled, close-to-nature climatic conditions and to use a newly developed system for phenotyping with respect to VOC emissions. The access provider team was given the opportunity (i) to expand the experimental applications of SignalSCREEN to new plant species, new pathogens and additional plant tissues, (ii) to apply in ExpoSCREEN the newly developed VOC phenotyping system (VOCSCREEN) for multi-stress responses of tomato, as well as (iii) for collaboration with new user groups in all installations.

Authors/Teams involved:

- SignalSCREEN: Mrs. Marion Wenig (technician), Dr. Miriam Lenk (scientist), Dr. Uta von Rad (scientist), and Dr. A. Corina Vlot-Schuster (principal investigator) Expo-
- SunSCREEN: Mrs. Petra Seibel (technician), Mrs Anna Mateeva (Team Assistant), Dr. J.B. Winkler (principal investigator), Prof. J.-P. Schnitzler (principal investigator).

Table of contents

1. Overview of TNA users projects realized in TNA HMGU

1.1.1. Installations

SignalSCREEN

This installation quantifies fluorescence from fluorescent proteins or dyes at the microscopic level. To this end, a spinning disc (confocal) microscope is used to image live plant seedlings or detached leaf or root tissues, which are loaded onto the wells of 96-well (Figure 1) and other multi-well plate formats. The microscope is loaded by using a robotic arm, which is responsible for exchanging the plates as the microscope analyses one plate after another during runs. Fluorescence intensities are measured at 5-10x resolution, normalised to those of control samples, and ranked as relative fluorescence units (rfu). Subsequently, the results of these automated analyses can be exported as excel sheets, ranking the wells of the different multi-well plates, which are included in an individual run, from high to low rfu. At HMGU, as part of its focus on analysing plant pathology-related phenotypes, the SignalSCREEN installation is routinely used to phenotype plants for their susceptibility to different pathogens. These can include fluorescently marked pathogens (using e.g. pathogenic organisms, which have been genetically modified to encode fluorescent proteins, such as GFP). In the frame of EPPN2020 most accesses were performed using fungal pathogens, which were visualised by staining fungal hyphae with the fluorescent dye 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM-DA; Lenk et al., 2018, 2019). In both of these approaches, higher rfu values are indicative of an elevated susceptibility of the plants to the pathogen used. Additionally, one access experiment phenotyped the accumulation of defense-active calcium ions after pathogen infection of different plant accessions, which had been genetically modified to encode a fluorescent calcium reporter. Here, higher rfu values correspond to elevated calcium levels in the cells, and potentially more resistant plant phenotypes.

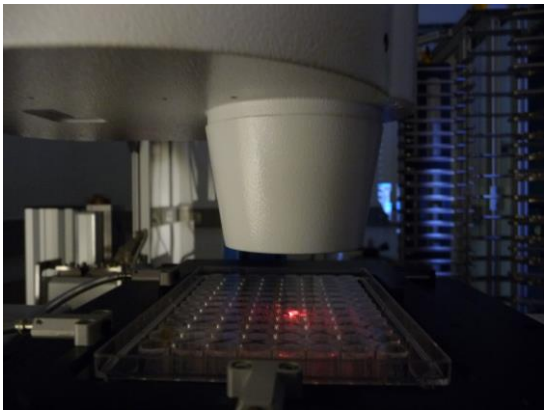

Figure 1 SignalSCREEN imaging the wells of a 96-well plate in the red fluorescent range.

ExpoSCREEN

The ExpoSCREEN comprises four walk-in exposure chambers (3.4 m long x 2.8 m wide x 2.5 m high) with controlled environmental conditions for air temperature (-20 °C to +45°C), relative air humidity (<10% to 90%), as well as light intensity and quality in the solar spectrum up to an intensity of 1000 µmol m^{-2} s^{-1} PPFD (Photosynthetic Photon Flux Density), and UV-B (280-315 nm) up to 1.2 W m^{-2}. In addition, plants can be exposed to variable atmospheric conditions, such as CO_2 (incl. sub-ambient concentration), ozone, NO_x, as well as other trace gases (including stable isotope labeling). Each ExpoSCREEN chamber can be optionally equipped with four temperature-controlled root containers (from -15°C to +25°C) to maintain soil temperature below (or above) air temperature. Installation of smaller, UV-transparent acrylic glass cuvettes on top of each root container enables subdivision of each walk-in chamber into four sub-chambers (area approx. 0.6 m^2, height above ground 1 m, depth of root container approx. 0.5 m). The sub-chambers allow individual control of the gas atmosphere (see above) under the same climatic conditions. In addition, the canopy gas exchange (CO_2, H_2O) of each sub-chamber can be measured. On-line PTR-MS (protein transfer reaction mass spectrometry) analysis allows dynamic monitoring of volatile organic compound (VOC) emissions. Also, a system consisting of 24 individual whole-plant gas exchange cuvettes can be installed in a walk-in chamber for phenotyping photosynthetic gas exchange and VOCs

(VOC-SCREEN). The ExpoSCREEN facility allows phenotyping of crops (e.g. wheat, barley, etc.), vegetable plants (e.g. tomato, potato, and soybean) and small woody plants (e.g. poplar, conifers, beech etc.).

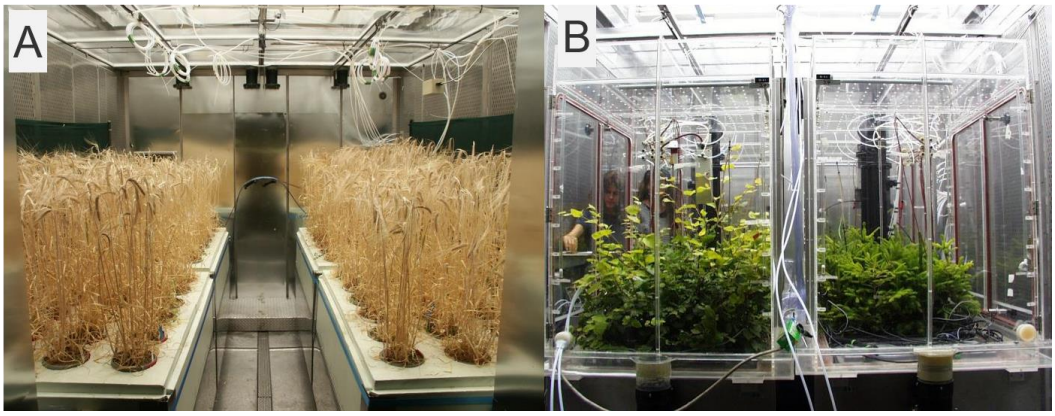

Figure 2: ExpoSCREEN, A: barley cultivars growing in a walk-in chamber, B: chamber equipped with sub-chambers.

SunSCREEN

This facility includes three solar simulators, two medium-sized chambers, each with a space of 1.4 m long x 1.4 m wide x 1.0 m high for plant cultivation, and a smaller chamber with a 1.2 m x 1.2 m x 0.3 m growing space. The environmental conditions can be controlled in the range of 15 to 30°C air temperature and 20 - 90% relative humidity. Light simulation takes place in the solar spectrum. The intensity of photosynthetically active radiation (PAR, 400-700nm) reaches up to 2000 µmol m^{-2} s^{-1} PPFD and in the UV-B range (315-320 nm) an intensity up to 3 (6) W m^{-2} can be applied. The size of the chambers allows phenotyping of model plants such as *Arabidopsis thaliana*, but also phenotyping of a wide variety of crops especially at the young stage such as cereals (e.g. barley, wheat, rice), vegetables (e.g. peppers, tomatoes), grapevines, pharmaceutical plants (e.g. peppermint, Arnica) or even small tree saplings (e.g. poplar, spruce, beech). In a typical SunSCREEN experiment, different plants or genotypes are exposed to different light qualities and amounts, e.g. to analyse the UV-B photoreceptor in plants or primary and secondary metabolites.

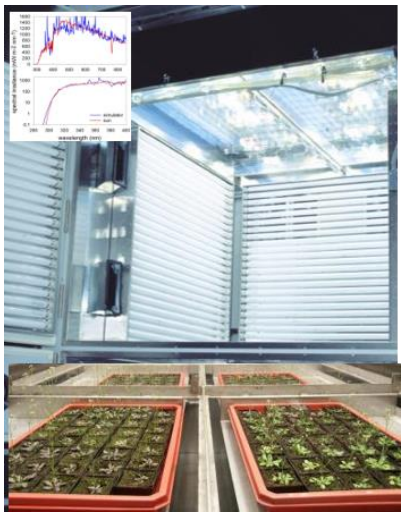

Figure 3: SunSCREEN chamber showing the lamp chambers set-up with water-filter and Arabidopsis genotypes. The insert shows the spectral conditions compared to natural irradiance.

1.1.2. User projects

	SignalSCREEN	ExpoSCREEN	SunSCREEN
Min. quantity of access units to be provided according to the DoA:	11	360	180
Total number of access units (sum of access units in the table):	11	446	162

Installation	Project title	Project acronym	Description about the experiment	Coordinator	Already used installation	Nature of the access unit*	Number of used access units during the project	(Potential) paper	How many people was trained by this procedure ?
SignalSCREEN	Role of DNA methylation variation in barley powdery mildew resistance	MethMildew	120 natural accessions of barley were phenotyped for their susceptibility to powdery mildew.	Bradley Till	No	(microscopy) RUN	3	(Yes)	Due to high travel costs between Chile and Germany, this experiment was performed without user present.
SignalSCREEN	Quantification of progression of fluorescently labelled *Phytophthora cinnamomi* in different genotypes of Castanea spp., using SignalSCREEN installation	CastaneaRoot-Phenotyping	6 genotypes of chestnut (2 parental lines and 4 hybrids) were phenotyped for their susceptibility to the root pathogenic fungus *Phytophtora cinnamomi* at four time points after infection.	Rita Costa	Yes	(microscopy) RUN	2	(?)	Due to COVID19-related travel restrictions, this experiment was performed without user present.

SignalSCREEN	Quantification of the progression of powdery mildew in genome-edited grapevine plants using SignalScreen installation	VitisMLO-Phenotyping	5 genotypes of grapevine (*Vitis vinifera*) were phenotyped for their susceptibility to powdery mildew at 5 time points after infection; this experiment was run in parallel in two environmental conditions using the SunSCREEN installation.	Claudio Moser	No	(microscopy) RUN	2	Yes (manuscript in preparation)	Due to COVID19-related travel restrictions, this experiment was performed without user present.
SignalSCREEN	Investigating Ca2+ signaling in priming and Systemic Acquired Resistance	Ca^{2+} signal in priming	Calcium-induced fluorescence was quantified by using a Ca^{2+} reporter transgene in 8 *Arabidopsis thaliana* genotypes and in *Nicotiana benthamiana* plants undergoing virus-induced gene silencing (VIGS; 10 constructs) to phenotype calcium fluxes in response to infection of the plants with *Pseudomonas.*	Keiko Yoshioka	No	(microscopy) RUN	2	(Yes)	Due to COVID19-related travel restrictions, this experiment was performed without user present.
SignalSCREEN	Assessment of downy mildew development in grapevine plants exposed to bioactive volatile organic compounds	DownyGrape	Grapevine (*Vitis vinifera*) plants exposed to 4 different chemical treatments were phenotyped for their susceptibility to the downy mildew pathogen *Plasmopara viticola* in 6 replicate experiments.	Michele Perazzolli	No	(microscopy) RUN	2	(Yes)	1 PostDoc from the user group was trained at the facility.

ExpoSCREEN	The effect of tropospheric ozone and nutrient availability on *Glycine max* and *Triticum aestivum*	ETON	Three wheat cultivars growing under simulated Mediterranean climate conditions were phenotyped regarding their response to 4 different ozone levels and 2 nitrogen fertilization regimes.	Melissa C. Chang Espino	No	1 day x 1 chamber	146	(Yes?)	1 PhD student was trained at the facility
ExpoSCREEN	Can moderate drought alter the content of volatile organic compounds (VOCs) of tomato cultivars affecting their susceptibility to herbivory?	Pheno-TOMVOC	Four tomato varieties (*Solanum lycopersicum*) were phenotyped with a focus on their VOC emission profiles under insect attack (using pipecolic acid as a proxy) and drought stress using the VOCSCREEN system installed within an ExpoSCREEN chamber.	Joan Llusia	No	1 day x 1 chamber	110	(Yes)	1 PhD student was trained during the whole experiment, and 1 PostDoc and 1 Senior Scientist during a short-term visit at start of the experiment
ExpoSCREEN	Phenotyping tomato lines with mutations in HT1 kinase in ExpoSCREEN platform	Future Tomato	Two lines of tomato (*Solanum lycopersicum)*, wild type and a genetically edited line defective in HT1 kinase, were grown for 21 days under ambient or elevated (+600 ppm) CO_2 concentrations and phenotyped regarding traits such as growth, stomata density, stomatal conductance and photosynthetic yield (PSII).	Hannes Kollist	Yes	1 day x 1 chamber	126	(Yes)	2 PhD students were trained at the facility

ExpoSCREEN	Functional characterization of downy mildew control mechanisms mediated by volatile organic compounds in grapevine	GrapeVOC	Grapevine plants (*Vitis vinifera*) adapted to an average climate typical of the region around Trento for one week were first exposed to 4 different chemical treatments, then infected with downy mildew (*Plasmopara viticola*) and phenotyped for susceptibility to this pathogen during the subsequent week.	Michele Perazzolli	No	1 day x 1 chamber	64	(Yes)	1 PostDoc from the user group was trained at the facility.
SunSCREEN	Evaluation of the susceptibility to powdery mildew of genome-edited grapevine plants in different environmental conditions using SunSCREEN installation	VitisMLOPhenotypingEnv	5 genotypes of grapevine (*Vitis vinifera*) were cultivated in the SunSCREEN i) mimicking the climatic conditions recorded in June 2019 in a local vineyard in northern Italy and ii) under a +3K temperature regime. Plants were infected with powdery mildew and their susceptibility to the pathogen was phenotyped at 5 time points after infection using the SignalSCREEN installation.	Claudio Moser	No	1 day x 1 chamber	102	Yes (manuscript in preparation)	Due to COVID19-related travel restrictions, this experiment was performed without user present.
SunSCREEN	Exploiting UV-B radiation to improve cold tolerance in bell pepper seedlings	FLAV-UP	Three bell pepper cultivars were phenotyped to investigate the priming potential of UV-B exposure for tolerance to subsequent cold stress.	Gyula Czégény	No	1 day x 1 chamber	60	(Yes)	1 PostDoc and 1 Senior Scientist from the user group were trained at the facility.

** Access units describe how accesses are calculated, typically 1 day x 1 pot, 1 season x 1 microplot etc.*

2. TNA projects

2.1.1. TNA projects description

ETON

The project aimed to study the combined effect of increasing ozone concentration and different nitrogen fertilisation systems on quality of soybean (*Glycine max*) and wheat (*Triticum aestivum*) varieties. As the user had not received the planned soybean seed, the experiment was carried out instead with three wheat varieties of different ozone sensitivity (traditional/tolerant, modern/tolerant and modern/resistant). Plants were grown in 2 ExpoSCREEN chambers, each with 4 sub-chambers, for 73 days under climatic conditions from Spanish wheat growing areas. To accelerate the growth period so that the plants reach flowering within the experimental timeframe, the average hourly climate parameters of 2 weeks were simulated within 1 week. The plants were exposed to 4 different ozone regimes (20, 40, 60 and 80 ppbv) and 2 fertiliser treatments (100 and 200 kg ha^{-1}), divided into 4 applications every fortnight (n=5 per treatment). Non-invasive measurements of ozone damage, plant size, photosynthetic activity, and epidermal pigment indices as well as sampling for biomarkers and stable isotopes were repeated regularly, followed by a final harvest and biomass determination. The traditional variety was found to be less ozone sensitive in terms of biomass while the more productive modern varieties were more sensitive to ozone. A compensating effect of higher nitrogen fertilisation could not be detected in preliminary data analyses. However, the data is under further analysis as part of the doctoral thesis of the user. During the experiment, Melissa Chang Espino (PhD student) was trained in the use of a photo station and various devices for non-invasive measurements of leaf physiological traits in the ExpoSCREEN.

PhenoTOMVOC

The aim of this project was to phenotype 4 tomato cultivars (*Solanum lycopersicum*) with regard to their response to abiotic stress (drought) in interaction with biotic stress (insect infestation). As neither aphids nor nematodes were available for biotic stress treatment at the time of the experiment, pipecolic acid (PA) was used as a proxy for insect attack. Two-week-old plants were grown for 2 weeks in ExpoSCREEN (13 h of daylight, 27/20ºC day/night). To explore the effect of the abiotic and biotic stresses on terpene production in leaves and roots of tomato, the VOCSCREEN system was used. After application of PA or MOCK treatment, plants were transferred to the VOCSCREEN cuvettes where +/- drought stress was started. Whole plant photosynthetic gas exchange (using IRGA) and volatile organic compound (VOC) emissions (using PTR-ToF-MS) were monitored on-line for 7 days. In addition, above- and below-ground VOC samples were taken for subsequent GC-MS analysis. Before and after cultivation in VOCSCREEN, images of each plant were taken in a photostation and total leaf area was determined. At final harvest, plant fresh weight and root volume were determined. Leaf and root samples were taken for metabolome analyses and root exudates were collected for organic carbon and metabolome analyses. The data analysis is not yet completed. Preliminary results indicate significant differences between the cultivars in terms of their sensitivity to biotic and abiotic stresses. The user group plans to publish the results in SCI journals.

Kaijun Yang (PhD student in the user group) was trained in conducting experiments in the ExpoSCREEN and VOCSCREEN and in plant imaging with a photo station. Dr. Catherine Preece (PostDoc) and Dr Joan Llusia Bennet (Senior Scientist) were trained in performing experiments with the ExpoSCREEN and VOCSCREEN.

MethMildew

The aim of this project was to exploit 120 *Hordeum vulgare* (barley) accessions from the USDA core collection to test if their susceptibility to the barley-specific powdery mildew fungus *Blumeria graminis* f. sp. *hordei* could be correlated to DNA methylation of regions of the barley genome. Prior to this EPPN2020 project, genotyping and climate-of-origin data had been

compiled for these 120 natural accessions of barley (Muñoz-Amatriaín et al., 2014); additionally, genome-wide DNA methylation data had been generated by the user group (unpublished). In this project, we analysed the susceptibility of the different barley accessions to powdery mildew. To this end, powdery mildew hyphae were stained with DAF-FM-DA and quantified on SignalSCREEN. The data for 113 of the tested accessions was of sufficient quality for further analysis. This analysis revealed correlations between powdery mildew susceptibility and both barley growth habit and the climate of the region, from which the accessions originated. Further analysis of the data is in progress, and the user group plans to include the data of this EPPN project in one peer-reviewed research publication addressing a possible role of DNA methylation in barley disease resistance.

CastaneaRootPhenotyping

This project aimed to assess the susceptibility phenotype of 4 hybrid *Castanea spp.* (chestnut species) and their parental lines *Castanea sativa* and *Castanea crenata* to the root phytopathogenic fungus *Phytophtora cinnamomi.* This project was part of a larger initiative to breed for chestnut species with enhanced resistance phenotypes to this particular fungus, which is threatening the European chestnut population. For this reason, the user group performed controlled crosses between the European susceptible variety *C. sativa* and the Asian chestnut *Castanea crenata*, which is resistant to *P. cinnamomi.* Four of the resulting hybrid lines and both parents (as controls) were analysed for their susceptibility to *P. cinnamomi* on SignalSCREEN. First, the access provider team learned how to prepare and inoculate chestnut roots, before the final experiment could be executed. Exchange between the user group and the access provider team was ensured by multiple online meetings, which were continued after the experiment to analyse and discuss the results. Because fluorescently labelled fungus was not viable, chestnut roots were inoculated with unlabelled *P. cinnamomi.* Subsequently, root preparations were stained with DAF-FM-DA to visualize fungal hyphae, and analysed on SignalSCREEN at 4 different time points after inoculation (0, 1, 3, and 7 days post-inoculation). By tracking the fluorescence on the roots of individual plants over the course of the different time points, the experiment revealed that three hybrid lines had intermediate phenotypes (between resistant and susceptible), whereas one hybrid line was similar to the susceptible parent/control line. The experiment thus showed that it is possible to breed for enhanced resistance against *P. cinnamomi* in chestnut. The users plan on including the SignalSCREEN data in one peer-reviewed research publication after further experiments reveal the nature of the enhanced resistance in the 3 hybrid lines with intermediate phenotypes.

VitisMLOPhenotyping / VitisMLOPhenotypingEnv

The objective of this project was to evaluate the susceptibility of four grapevine (*Vitis vinifera*) genotypes to the grape powdery mildew fungus *Erysiphe necator* under 2 different environmental conditions, present and expected future temperatures. Among the genotypes was one control (parental) line and 3 mutants, which had been generated by CRISPR-Cas_9-mediated genome editing. To ensure proper environmental simulation and phytopathology, this experiment was performed using 2 installations: 2 SunSCREEN chambers for VitisMLOPhenotypingEnv and SignalSCREEN for VitisMLOPhenotyping. In 1 SunSCREEN chamber, climatic conditions were simulated based on measured parameters at the user's site in June 2019. In the other chamber, the predicted temperature increase due to climate change was simulated with ~3°C higher temperatures, but not exceeding a maximum of 30°C. Plants were acclimatised to the SunSCREEN environment for 1 week, gradually increasing the daily maximum light intensity at plant level from 250 to 700 µmol m^{-2} s^{-1} PPFD. Immediately after the acclimatisation phase, 5 plants per genotype and chamber were inoculated with powdery mildew. Subsequently, powdery mildew hyphae were stained with DAF-FM-DA at 4 different time points (9, 10, 13, and 16 days post inoculation; at 13 days, young and old leaves were harvested separately). In a second experiment, 1 additional time point (7 days post inoculation) was added. While differences in the susceptibility phenotypes of the different grapevine lines were not observed when comparing the different temperature conditions or leaf age, one mutation (H34, see section 2.1.2. below) caused reduced propagation of powdery mildew

hyphae over time. Thus, this particular genetic modification promoted grapevine resistance to the powdery mildew pathogen. This is the first demonstration of whole plant resistance of grapevine against *E. necator* due to a CRISPR-Cas_9 gene edit. The user group in cooperation with the access providers at HMGU are currently preparing a manuscript for a peer-reviewed journal. The access provider teams of SignalSCREEN and SunSCREEN will support this manuscript with additional experimental replicates (in October and November 2021), during which Dr. Lisa Giacomelli (scientist in the user group) who could not come during the TNA experiments due to COVID19-related travel restrictions, visits the installations and is trained in data analysis.

Ca2+ Signal in Priming

This project assessed a possible importance of calcium ions (Ca^{2+}) in plant defense reactions against pathogens. To this end, *Arabidopsis thaliana* and *Nicotiana benthamiana* plants carrying transgenes encoding the fluorescent Ca^{2+} sensor GCaMP3 were used (DeFalco et al., 2017). GCaMP3 fluorescence was measured using the SignalSCREEN parameter settings as for GFP and DAF-FM-DA, which had been supported by preliminary experiments prior to the Ca^{2+} Signal in Priming application. In this experiment, high rfu values correspond to high Ca^{2+} levels in plant cells. The *Arabidopsis thaliana* plants were treated with pipecolic acid, a known defense metabolite (Lenk et al., 2019; Vlot et al., 2021), and subsequently inoculated with pathogenic *Pseudomonas spp.* Among 8 *A. thaliana* genotypes (2 wild type accessions and 6 mutants), pipecolic acid-induced priming of Ca^{2+} fluxes was highly variable. For this reason, further experimentation in this project focused on *N. benthamiana.* Parallel experiments confirmed differences in Ca^{2+} fluxes in two of the *A. thaliana* mutants, and the user group is preparing a manuscript to be published in a peer-reviewed journal.
In *N. benthamiana,* virus-induced gene silencing (VIGS) was used to downregulate transcript accumulation of 10 target genes. This experiment aimed to evaluate a possible role of these genes in defense-associated Ca^{2+} regulation. After VIGS, the plants were inoculated with pathogenic *Pseudomonas spp.,* and the resulting GCaMP3 fluorescence was measured on SignalSCREEN 24 hours later. Data from 6 biologically independent replicates of this experiment (including 4 plants per replicate, analysing 5 leaf discs per plant) revealed 3 genes with (possible) functions in the regulation of defense-associated Ca^{2+} fluxes. As these included the positive control gene (which had been blinded for the access provider team) the user group and access provider expect to include the other 2 genes in future manuscript(s).

FutureTomato

The project aimed to phenotype water usage, biomass production and overall plant growth of wild type (WT) and genetically edited (HT1) tomato plants (*Solanum lycopersicum*) with altered stomata regulation under elevated CO_2. Plants (60 of each genotype) were grown in 2 ExpoSCREEN chambers, each with 4 sub-chambers, under ambient or elevated CO_2 concentration (ambient +600 ppmv) for 21 days. During the experiment, the canopy gas exchange of each sub-chamber was measured continuously and, in addition, weekly manual measurements of leaf gas exchange and PAM-Chl fluorescence were made and the plants were imaged in a photostation. At the final harvest, samples for biochemical analysis were taken and above-ground biomass was determined from 2/3 of the plants while 1/3 of the plants were kept in the greenhouse to grow fruits for fruit quality analyses (performed by the access provider). The experiment was repeated 3 times independently. The data analysis is not completed yet as the experiment took place in summer 2021. The user group is preparing a publication.
During the experiment, Triinu Arjus and Kaspar Koolmeister (PhD students in the user group) were trained in conducting experiment in the ExpoSCREEN, in the use of a photo station and various devices for non-invasive measurements of physiological traits at leaf level.

FLAVup

This project aimed to analyse 3 pepper cultivars that differed in leaf phenolic patterns regarding their susceptibility to cold stress and the potential of UV-B exposure to prime plants to be more

tolerant to subsequent cold stress. Plants were grown from seeds during 6 weeks in one SunSCREEN chamber under controlled climatic conditions (14h/10h day/night, temperature 25/20°C, rel. humidity 50/70%, PAR ~300-400 µmol m^{-2} s^{-1}, no UV-B). After 6 weeks, half of the plants of each cultivar were transferred into a second SunSCREEN chamber, and exposed for 5 days to the same environmental conditions but with UV-B radiation for 6 h daily (~ 6 kJ m^{-2} d^{-1} $UV\text{-}B_{be}$). The other group served as a control. At the end of the UV-B exposure, 10 plants per cultivar and UV-B treatment were sampled for biochemical analysis. The remaining plants from each UV-B treatment were divided into 2 groups: plants kept under 25/20°C and plants exposed to cold stress (15/10°C). The cold treatment lasted another 5 days. During the 10 days of UV-B and cold treatment, plant growth was monitored using a photostation, and leaf pigments and PAM-Chl fluorescence were measured non-invasively at regular intervals. At the end of the experiment, all plants were sampled and frozen for further laboratory analyses. The experiment was performed in September 2021, so data analysis has not yet been completed. The users plan to publish the results in peer-reviewed journal.
During the main experiment, Dr. Gyula Czégény and Prof. Éva Hideg from the user group were trained in performing experiments in SunSCREEN and in the use of the photostation.

GrapeVOC / DownyGrape
This project phenotyped the influence of 3 different volatile organic compounds (VOCs) on the susceptibility of grapevine (*Vitis vinifera*) to the downy mildew pathogen *Plasmopara viticola.* The VOCs, which were tested, had been identified by the user group in the headspace of *P. viticola*-inoculated grapevine plants and in emissions of plant beneficial fungi (*Trichoderma spp.*). In order to obtain reliable whole-plant phytopathology data, the plants were grown in precise, realistic and reproducible environmental conditions (ExpoSCREEN). After 7 days of acclimatization to the climatic conditions in the ExpoSCREEN (day length 16 h, temperature 28°C day / 25°C night, rel. humidity 65% day / 85% night, PAR approx. 750 µmol m^{-2} s^{-1}), plants were exposed to the VOCs or to a MOCK treatment for two days. During the second day of the treatment, half of the plants were additionally inoculated with *P. viticola*. Subsequently, the plants were kept in separate sub-chambers in the ExpoSCREEN for a further 6 days according to their VOC or pathogen treatments, so that no mutual interference via VOC emissions resulting from the treatments could take place. The experiment was performed 6 times in a row (n=6 biologically independent replicates). Both at day 1 and 6 after inoculation, plants were non-invasively analysed for physiological traits using optical devices, such as photosystem II capacity (Fv/Fm), stomatal conductance and epidermal flavonoid accumulation (flavonols, anthocyanins). In addition, leaf samples were taken for further transcriptome and metabolome analyses (GrapeVOC). Subsequently, *P. viticola* propagation on the leaves was assessed on SignalSCREEN at day 6 post-inoculation. To this end, leaf discs from the inoculated leaves were stained with DAF-FM-DA, and rfu values were obtained by normalizing the fluorescence signal in *P. viticola*-inoculated leaf discs to that in the respective uninoculated controls. Preliminary data analysis revealed that exposure of whole grapevine plants to VOCs altered leaf physiological traits. In addition, 2 of the VOCs used in this project, reduced *P. viticola* propagation on the grapevine leaves and are thus candidate compounds for integration in future, durable crop protection strategies. The analysis of the transcriptome data (RNA-Seq) is planned for the near future and the user group aims to publish the data in due time.
This project was performed in late summer 2021, allowing user presence at the installations. Over the course of the experiment, Dr. Valentina Lazzazara (scientist in the user group) was trained at using the optical devices for non-invasive measurements at leaf level in the ExpoSCREEN and at performing experiments and analysing data on SignalSCREEN.

2.1.2. Selection of One exemplary project

Propagated for centuries for the purpose of human consumption, grapevine constitutes one of the oldest domesticated crops, in particular in warmer regions of the world. Nevertheless,

grape production is highly dependent on the use of chemical fungicides to reduce crop losses due to pathogens, such as *Erysiphe necator*, the causal agent of grapevine powdery mildew. At the same time, grapevine has remained relatively inaccessible for biotechnology-based breeding techniques avoiding major advances in improving the resistance of grapevine against powdery mildew. More recently, the user group of **VitisMLOPhenotyping / VitisMLOPhenotypingEnv** at Fondazione Mach (San Michele all' Adige, Italy) and others have established modern genome editing in grapevine, which opens up a whole new spectrum of breeding possibilities in this crop plant.

By using clustered regularly interspaced short palindromic repeats (CRISPR) in combination with CRISPR-associated nucleases (Cas) genome editing, the user group introduced mutations into the grapevine genome which were aimed at reducing the susceptibility of the plants to powdery mildew. The mutants analyzed in this experiment, included 2 single and 1 double mutant (including both of the single mutations present in the other lines). The target genes are members of the same gene family and are currently included in a running patent application. To support a possible future necessity of these and/or similar mutations in grapevine resistance to powdery mildew, the infection experiment of the mutants was conducted in both current and future climates. The treatment 'cool' indicates climate conditions simulated in SunSCREEN that were recorded at the user's location in Trentino, Italy, in June 2019, while for the ' warm' treatment the temperatures were set 3°C higher. Powdery mildew inoculation resulted in visible powdery mildew hyphae appearing on the leaves between 7 and 9 days post-inoculation. SignalSCREEN was then used to phenotype the progression of the infection from 7 (experiment 2) or 9 (experiment 1) days until 16 days post-inoculation. While the single mutants displayed a tendency of reduced pathogen propagation as compared to the wild type control, the double mutant displayed significantly less powdery mildew propagation over the course of the experiment. The data thus confirm that the CRISPR-Cas-induced mutations reduced the susceptibility of the grapevine plants towards powdery mildew. This is the first observation of such a phenotype in these genotypes at the whole plant level, supporting a possible future application of this approach to improve powdery mildew resistance in grapevine.

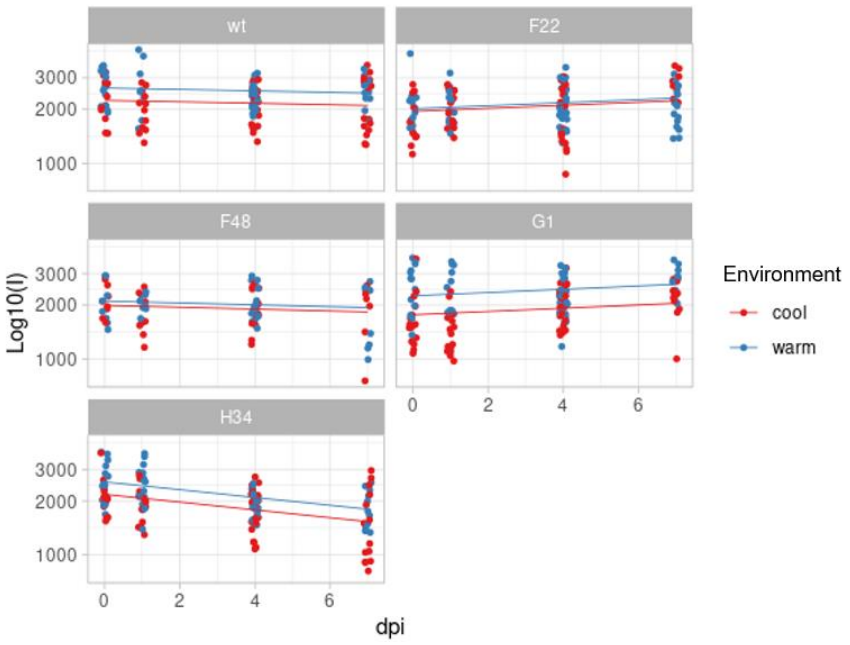

Figure 4: Powdery mildew development over time on grapevine wild type (wt) and genome-edited single (F and G) and double (H) mutant plants at cool (red) and warm (blue) temperatures. Plants were kept in SunSCREEN during the experiment, and data represent fluorescence units derived from SignalSCREEN analysis. Units X-axis: 0 = 9 days post-inoculation; 2, 4, and 6 on axis correspond to days 11, 13, and 15. The double mutant H34 is the only genotype, which displays a reduction in the infection values, indicating reduced powdery mildew propagation and thus enhanced resistance of this genotype to the pathogen.

3. Reflection on results of the TNA programme

Overall, the HMGU access providers had very good experiences (see main result of the executive summary). Many users adopted the providers' suggestions in experimental design and were willing to be present at the installation during the experiment despite the COVID-19 pandemic to perform the measurements themselves, and postponed their accesses accordingly. However, it became apparent (i) that more time should be allocated to the users' experimental design, (ii) that the users need to ensure that they have done the necessary preparatory work or obtained the required material (e.g. sudden lack of insects or seeds at the time of the experiments) or (iii) that they should formulate their requests more clearly in advance as to which additional measurements they still need in the provider laboratory. This could be improved in future access programs by reducing the frequency of calls and increasing the subsequent period for experiments.

4. References

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