KliWiResse

Klima-Widerstandsfähige Rebsorten zur Sicherung des Ertrags

Newsletter #3 (June 2023-September 2023)

UPCOMING EVENTS

November 30th 2023

KliWiResse Project Meeting II, Julius Kühn-Institut (JKI)-Bundesforschungsinstitut für Rebenzüchtung, Geilweilerhof, 76833 Siebeldingen

https://www.julius-kuehn.de/

Project meetings:

07.07.2023: The first project meeting of KliWiResse was held at FiBL Frick, Switzerland (Ackerstrasse 113, 5070 Frick). The Delegates from all project partner groups attended the meeting, presented the latest results, and enhanced the collaboration. Besides sharing the latest exciting results among the partners, the participants discussed strategies for enhancing direct collaboration among the groups.

ScreenSYS at Freiburg, Germany aims to develop a double haploidisation protocol for grapevine varieties. This procedure is based on microspores, the progenitors of pollen cells, which can be reprogrammed to develop plantlets with doubled haploid genomes. After coordination with several projects and associated partners (WBI, JKI, FiBL, KIT), ScreenSYS received flower material from different genotypes and microspore expert Dr. Qiuju Yu established the basic protocol parameters (optimal flower development timing, and micropore isolation procedure). For further experiments, the genotype Calardis Blanc was selected as it will be available from most of the project and associated partners which is required to warrant continuous plant material availability for protocol development. Using material from JKI and FiBL, ScreenSYS identified the best culture medium and several conditions which enhanced the microspore viability in culture. With the next available material, protocol development will continue by applying various stress treatments and chemical regulators to induce microspore cell divisions. Another contribution of ScreenSYS in the project is the development of a screening system with leaf cells (protoplasts) from different grapevine varieties to investigate cell-specific differences in their response towards heat and drought. Dr Ralf Welsch and colleagues at ScreenSYS have successfully developed a protocol for the isolation of viable cells (protoplasts) from leaves of various grapevine varieties. ScreenSYS protoplast expert Amir Moradi identified suitable heat application conditions to screen for resilience differences of leaf cell viability towards increased temperatures. Twelve genotypes with known differences in their heat resistance were processed and cell viability was determined using viability dyes. An automated cell recognition and cell counting routine is currently developed to quantify the experiments and classify the genotypes according to their response.

KIT at Karlsruhe, Germany has successfully developed a novel method to assess the stress tolerance characteristics of grapevine varieties in a non-invasive way. They have used excised leaf discs as a proxy for grapevine plants and subjected them to various stress treatments. Ms Paula Venzke (Master student), Mr Ruslan Eliseev and Mr Ulaş Cönkeroğlu (Bachelor students) have worked on establishing the core protocols for leaf disc stress assays in grapevine, which offer a great advantage of conducting sophisticated experiments in a relatively simple way. In parallel, a comparative time-course study using Riesling (heat-susceptible) and the sylvestris accession Hördt 29 (heat-tolerant) has been conducted, measuring numerous physiological parameters (also using the new non-invasive MiniPAM device to characterize photosynthetic parameters). The design was improved over that from last year. Samples will be tested for gene expression, but also for establishing the jasmonate profiles (in cooperation with IBMP). Further insight into the molecular mechanisms underlying heat resilience in Hördt 29 has been discovered, for instance, a superior non-photochemical quenching of the photosynthetic system. Expression of specific heat-shock proteins, along with genes involved in redox homeostasis can be used to predict heat resilience. There is a good match between the data obtained from the leaf-disc system and the plant system, showing that the leaf-disc system represents a good experimental

model for screening a larger number of genotypes. The core set of genotypes defined with the partner has already been screened successfully. The heat-sensitivity of Riesling has been confirmed, as well as the tolerance of Hördt 29. Interestingly, also Chardonnay performs well under heat stress. A carbon-isotope-based method has been set up to monitor stomata closure under field conditions. This is currently validated by monitoring rootstock varieties and different *sylvestris* accessions in the Botanical Garden of the KIT. If this works out, the method can later be used to map the crossing populations at JKI, but also the PiWi and rootstock collections at FiBL. The latest results from these experiments, showing the differences in leaf disc stress responses among different varieties, were presented at the meeting by Ms. Manasi Nabar and Ms. Paula Venzke. The hormonal and metabolite analyses are currently conducted during a research visit of Ms. Paula Venzke at the IBMP.

IBMP Strasbourg and KIT are working together on the metabolomic analyses of grape leaf samples to reveal the variety-specific stress pathway signatures. Heat stress treatments were performed at KIT and the collected samples were successfully transferred to IBMP, where they will conduct high throughput analyses using advanced metabolomic platforms with the help of Dr Thierry Heitz, Ms Dennise Beltran and other colleagues. Ms Paula Venzke from KIT is cooperating with the partners at IBMP for metabolomic analyses and will visit Strasbourg from September to October 2023. A targeted LC-MS/MS approach will first evaluate the quantitative profiles for stress hormones including various forms of jasmonate, abscisic and salicylic acids in riesling and Hördt 29 after a heat shock. Later on, the material will be submitted to non-targeted, high-resolution LC-MS/MS to identify metabolic signatures that may be associated with grapevine abiotic stress susceptibility or tolerance. Further development to investigate central metabolism behaviour by GC-MS can be foreseen.

JKI at Siebeldingen, Germany remains the major source of grapevine plant materials for other research groups to conduct various experiments. JKI has developed high throughput artificial intelligence-based tools for quantifying downy mildew disease severity and leaf hair quantification. Currently, Nagarjun Malagol and Dr Oliver Trapp are working on optimizing the established phenotyping pipeline to study the resistance of grapevine varieties to sunburn (heat and UV stresses). In addition, efforts are made to develop AI-based tools for sunburn quantification on berries. The AI tools created at JKI are capable of screening a large number of grapevine germplasm, providing crucial phenotyping data for quantitative trait locus analysis. Subsequently, in addition to the phenotyping data, genetic mapping and QTL analysis of sunburn resilience in the lab and field are performed at the JKI. Molecular markers developed in QTL regions will then enable the use of marker-assisted selection in breeding programs and to create novel sunburn-resilient grapevine cultivars. They can measure the berry size, shape, colour, and damage using image analysis and machine learning algorithms. They have plans to develop a web-based platform to share their data and tools with other partners. JKI will host the second project meeting on November 30, 2023.

The meeting also provided an opportunity for exchanging ideas, feedback, and experiences among the project partners and successfully advanced the project objectives to foster a productive and cooperative atmosphere.

FiBL at Switzerland is actively working on the grape cultivars at the institute´s vineyards screening for stress tolerance. After two heatwaves, disease and solar stress counts have been completed, and physiological and oenological analyses are underway. On pilot farms in the partner cantons of Basel-Landschaft (BL), Jura (JU) and Aargau (AG), disease and solar stress counts have been carried out, and analyses of leaf reserves and oenological parameters are in progress.

Interactions

1. KIT-ScreenSYS: Leaf material from the core set of genotypes was continuously transferred from KIT to ScreenSYS as well as inflorescences for the double haploidisation.

2. KIT-JKI: For the crossing population between Hördt 29 (heat resilient) and the vinifera variety Tigvoasa (male sterile, for crossing) established by JKI and KIT, genomes from the parents and selected individuals of their offspring were sequenced and integrated into the genome database GrapeKIT, which will allow to search for alleles for the genes already found to be linked with heat stress. The berries of this and other *sylvestris x vinifera* crossing populations established by KIT and JKI are integrated into the sunburn phenotyping platform at JKI.

3. KIT-IBMP: Material from the time course study with Riesling-Hördt 29 (from whole plants grown in the greenhouse) were sent to IBMP for extensive metabolite analysis. Ms Paula Venzke from KIT is working at the IBMP

for a few weeks to assist with the analysis. Material from a standardized experiment with leaf discs submitted to heat stress was also transferred and is awaiting analysis.

4. FiBL-KIT-JKI: Material from the FiBL PiWi collection has been sent to the partners and is included in the studies on heat stress and sunburn

5. KIT-WBI-ScreenSYS: Flower buds in defined stages have been sent from KIT to ScreenSYS. Meanwhile, a greenhouse-based system has been established at WBI that allows to provide ScreenSYS to the right floral stage throughout the year on a weekly basis.

6. **JKI-FIBL**: Evaluation of cultivars supplied by the JKI for solar stress and disease assessments have been carried out, and specific analyses of nutrient storage are in progress. Leaf discs are also being collected for the KIT team to determine $\delta^{13}C$. Calardis grape plants, a source of floral parts for ScreenSYS, were also assessed for disease, nutrition and solar stress.

6. JKI-FIBL:

7. JKI-IBMP: Material from crossing individuals (Hördt 29 x Tigvoasa, generated by KIT and JKI) and the Calardis Blanc x Morio Muscat generated at JKI has grown side by side in the JKI vineyard are being collected and will be sent to IBMP for hormonal profiling. The idea here is to use metabolomics as a readout of cumulative natural stress endured by the progeny during the summer.

Website:

KliWiResse's website has now been updated with the scientific portraits of project partners with a brief description of their research activities. A separate space will also be provided with simplified text for farmers´ and public awareness. Newsletters published in a timely manner has now a dedicated place on the website (https://kliwiresse.wine-science.eu/news.htm#start)

Newsletter:

Newsletter #3 with the updates on project meetings is expected to be out by the end of September 2023.

Flyers and roll-ups:

JKI and IBMP received the first roll-up posters during Project Meeting I held at FiBL, Frick, Switzerland. The posters for the remaining interested partners will be delivered during Project Meeting II at JKI, Germany.

Outputs:

Surveys: a questionnaire for winegrowers was developed in cooperation between KIT, FibL, and Weincampus Neustadt. It was already used for two polls by FibL (participants from Switzerland and France) and KIT/Weincampus (participants from France).

Public relation events:

- The project was presented by Peter Nick (KIT) to an international auditory in a presentation (“Viticulture versus Climate Change – together we will cope!”) and a panel discussion at the Summer School “Future of Life” at the Hochschule für Kunst und Gestaltung Karlsruhe 26.07.2023.
- At the JKI's day, open door 2023 at Siebeldingen, Nagarjun Malagol and Dr Trapp presented the project, giving over 1000 attendees a broad overview and outlining its aims for developing climate-resilient grape varieties. Additionally, flyers were given out and roll-ups were shown.

Media outreach (press release, TV/Radio programs):

Talks and seminars:

Symposia: The first event "Wine - Climate - Together We Can Do It" by KIT (Peter Nick) and Weincampus Neustadt (Maren Scharfenberger-Schmeer) was held on 13.06.2023 at the Weincampus Neustadt with young winegrowers from France and Germany. Flyers and roll-up shown at the Artenschutztag of the Zoo Karlsruhe 10.09.2023 (Michael Riemann).

Farmers' awareness: A farmer´s awareness program was conducted by FiBL on March 15th 2023. The activities related to KliWiResse were explained to the farmers by a team led by Hans-Jakob Schärer

Scientific achievements:

Latest scientific breakthroughs:

KIT: a comparative study on heat stress in plants of Riesling (sensitive) and sylvestris Hördt 29 (tolerant) was conducted (Master thesis Paula Venzke). The design was improved over the experimental campaign of 2022 including a longer acclimation period in the chamber at normal temperature. The plants were monitored non-invasively using a miniPAM system. This showed that the improved design yields more stable data. Moreover, the physiological parameters of Hördt 29 show better resilience over Riesling. Interestingly, the resilient Hördt 29 has a significantly higher activity of non-photochemical quenching, which might be a way to dissipate heat and to safeguard photosynthetic electron transport. Samples for gene expression analysis and metabolic analysis were generated. The metabolic analysis is currently evaluated at IBMP in the frame of a research visit by Paula Venzke. In parallel, a heat-tolerance screening system with leaf discs was developed (Bachelor thesis Ruslan Eliseev). To eliminate the effect of wounding, the leaf discs were acclimatised on agar in Petri dishes, before being exposed to a heat shock. This system worked well and allowed following early gene expression, confirming the patterns seen in the plant for the pair Riesling (sensitive) and sylvestris Hördt 29 (tolerant) even with respect to details. In particular hsp17, and hsp18 (cytosolic) were induced transiently at higher amplitude in Hördt 29, hsp20 (plastid) showed instead steady and slow increase, which was comparable, hsp22 (ER) displayed rapid and transient induction, which was more persistent in Hördt 29. The expression of ascorbate peroxidase decreased in heat in both genotypes from around 1-3 h like in plants by heat. However, dehydroascorbate reductase, the enzyme replenishing ascorbate showed higher ground levels in Hördt 29. Among the other antioxidant genes, the plastidic Superoxide Dismutase was decreased under heat stress in both genotypes, as well as the mitochondrial MnSOD1. Thus, the ascorbate (vitamin C) cycle might be crucial for heat tolerance, as well as heat shock proteins hsp17 and hsp22. Since the leaf-disc assay worked so well, it was used to compare the genotypes from the core panel that had been defined among the partners in winter 2022. Here, Riesling and Morio-Muscat turned out to be the most heat-susceptible varieties, while sylvestris Hördt 29 was performing superior to all competitors. Calardis Blanc was intermediate. Interestingly, Chardonnay showed a good heat tolerance as well and, thus, might be a white variety that could replace Riesling in the future. The leaf-disc assay was further used to monitor the redox status during the early phases of heat stress (ERASMUS student Ulaş Cönkeroğlu). A quantitative assay based on histochemical detection of superoxide (by Nitroblue Tetrazolium) and hydrogen peroxide (Diaminobenzimide). This assay was calibrated and detected that heat stress generates an early, transient rise in superoxide, which is later followed by the accumulation of hydrogen peroxide. This oxidative burst occurs earlier in Riesling, while Hördt 29 can buffer photosynthesis several hours more before it shows an oxidative burst. To compare the performance of different genotypes in the field, a carbon isotope-based method has been adjusted (PhD student Manasi Nabar). Since RubisCO, the enzyme fixing carbon dioxide, prefers one isotope over the other, the ratio of C_{13} over C_{12} reports, how long stomata were open (when they are closed, carbon dioxide from respiration has to be used, which differs in the ratio from ambient carbon dioxide). This approach allows to compare plants grown in the field side by side on a relative level. Susceptible plants will have their stomata closed longer than resilient plants. This method is currently applied to the grapevine collection at KIT, including a set of common rootstocks used in the Upper Rhine region. If the method is validated, it can in the next season be used to screen the crossing populations at JKI, but also the PiWi collections at FibL. A study on surface waxes in *V. sylvestris* revealed considerable diversity, whereby the wild genotypes accumulate more surface wax than commercial varieties. Surface wax is known to reflect heat and is therefore beneficial for heat tolerance in several crop species. In addition, we could show that genotypes rich in surface wax were partially resistant to Powdery Mildew (*Erysiphe necator*), a disease that over the last years has gained impact over the previously widespread Peronospora (*Plasmopara viticola*). This change is a consequence of climate change because Peronospora is favoured by cool and humid summers, while Powdery Mildew prefers warmer and drier summers as we see them now also in the Upper Rhine region. The reason for the partial resistance is

a delayed formation of the fungal appressorium which gives the plant more time to deploy defence responses. This study has been published in BMC Plant Biology.

Since many of the *sylvestris* genotypes also grow at JKI, they will be integrated into the sunburn phenotyping platform, because surface waxes on the berry skin are also relevant for resistance to Botrytis (connection with Wivitis).

JKI: The previously established sunburn phenotyping pipeline was optimized during the season to get close to the factual results. In addition, UV radiation will be considered for the set of laboratory-based experiments. Eventually, this will help us in building a more precise laboratory-based sunburn phenotyping pipeline.

The sunburn resistance of 100 genotypes from the JKI's germplasm was investigated. Whereas 35% of the genotypes have shown very high tolerance to sunburn and might be suitable as genetic resources for further crossbreeding activities. In addition, a mapping population of 'Morio Muskat' x COxGT2 (V. coignetiae × 'Gewürztraminer') was screened for sunburn resilience and the preliminary results have shown excellent segregation of the trait (sunburn), thus this population serves a potential population for quantitative trait loci analysis (QTL). Additionally, the Vitis sylvestris Ketsch accessions (, n=34), which are materials from a prior project partnership with KIT were screened using the sunburn phenotyping pipeline and have demonstrated good sunburn resilience. Furthermore, the biparental Tigvoasa x V. sylvestris Hördt 29 (resilient) population was screened for sunburn resilience and was generally highly tolerant.

A new high throughput image acquisition template was developed using an Axio Zoom stereo microscope which can capture around 2000 high-quality berry images per hour. Sunburn pre-treatment and post-treatment berry images were captured using the above template to develop an artificial intelligence (AI) based tool for sunburn severity quantification. The development of the CNN-based model is in progress.

Methods developed:

KIT: 1. Leaf disc assay to validate the degree of tolerance to heat stress. This assay recapitulates the observations in intact plants very well but requires much less time and space, such that it can also be used to screen numerous genotypes. Readouts are specific members of the heat-shock proteins. 2. Histochemical assay to measure specific reactive oxygen species as markers for oxidative stress in consequence of heat. The assay could be rendered quantitative based on a strategy using quantitative image analysis. 3. Carbon isotope-based assay to assess the stomatal history during a heat and drought episode in the vineyard. This assay is relative – one can compare different genotypes that had been grown side by side in the same vineyard. This assay will be transferred in the next season to partners JKI and FibL to screen the crossing populations and the PiWi collections. 4. Non-invasive monitoring of the heat stress *in planta* using the miniPAM system.

ScreenSYS: 1. Method for protoplast isolation from grapevine leaves and assessing viability development over time. 2. Method for isolation of microspores from grapevine inflorescences and identification of culture conditions to maintain high viability.

Results arising from collaborations:

KIT: leaf material and immature flower buds were delivered in a continuous flow to partner ScreenSYS for the protoplast and double haploidisation experiments. Material from the heat-stress experiments (both, plants, and leaf discs) has been delivered to partner IBMP for metabolic analysis, in particular jasmonates. These samples are currently being analysed by Paula Venzke from our lab in support of Ms Dennisse Beltran during a research visit at IBMP.

Manuscripts published:

KIT: Ge XS, Hetzer B, Tisch C, Kortekamp A, Nick P (2023) Surface wax in the ancestral grapevine Vitis sylvestris correlate with partial resistance to Powdery Mildew. BMC Plant Biology 23, 304