KliWiReSSe Klima-Widerstandsfähige Rebsorten zur Sicherung des Ertrags

Newsletter #1 (December 2022-February 2023)

UPCOMING EVENTS

Official kick-off meeting of KliWiReSSe

Expected to take place in January 2023

Project meetings:

The first project meeting of KliWiReSSe was held at KIT-BOT, Germany on 10th November 2022. The partners planned the interactions and exchange of materials and data.

The official kick-off meeting with all the associated partners will take place in January 2023. More information on this meeting will be disseminated by the end of December 2022.

An additional kick-off meeting conducted by Interreg Oberrhein took place on 15th December 2022 (10.30 AM to 15:30 PM) at 1 place Adrien Zeller, Strasbourg. Representation from all the cooperating partners is expected.

Interactions

Synchronising the genotypes: To ensure maximal synergy, partners agreed to include a core collection of genotypes throughout. A video meeting between FiBL, JKI and KIT on 12th December 2022 defined the commercial variety Riesling (heat-susceptible) and the wild *sylvestris* accession Hördt 29 (heat-tolerant) as the first priority for comparative mapping of stress markers. Reason: maximal difference in stress tolerance, Riesling is economically the most relevant cultivar in the region and has originated here, there exists already preparatory research on stress tolerance, and there are cell lines of both genotypes reflecting the stress tolerance pattern of the plant. A crossing population of Hördt 29 with Augster Weiß is established at JKI. Also in the priority group is the PiWi variety Calardis Blanc (high tolerance to sunburn) and the traditional Morio Muskat (low tolerance to sunburn) because a crossing population has already been established at JKI. The second priorities are the traditional varieties Chardonnay (heat sensitive, cell cultures available) and Pinot Blanc (Weißburgunder), Augster Weiß and *sylvestris* varieties Ketsch 53 and Ketsch 83 because crossing populations for those have been established at KIT. In addition, around five genotypes from the FiBL PiWi collection will be included (e.g., Johanniter, Cabernet Cortis, Solaris). Wood cuttings will be established and exchanged between FiBL, JKI and KIT in January-February 2023, depending on the weather conditions.

Exchange of materials:

1. KIT-ScreenSYS:

Since experiments on leaf material are not feasible during the winter season, the screening system at ScreenSYS will be adjusted using protoplasts from cell cultures. For this purpose, cell cultures for Chardonnay (heat-sensitive) and Hördt 29 (heat-tolerant) have been transferred from KIT to ScreenSYS.

2. JKI-KIT:

To map early stress markers and establish the signalling system for heat stress, cell lines with contrasting stress sensitivity are crucial. So far, at KIT the contrasting pair Chardonnay – Hördt 29 has been investigated. However, in the context of synchronisation, the cell culture of Riesling as a sensitive genotype would allow for more synergy. Such a cell line was available at JKI and has now been transferred to KIT.

3. KIT-IBMP:

A comparative heat-stress experiment with plants from Riesling (heat sensitive) and Hördt 29 (heat tolerant) has been conducted at KIT and from this experiment, leaf material has been collected and transferred to IBMP for extraction and metabolic analyses.

4. JKI-ScreenSYS:

For ScreenSYS to have more available plant material throughout the year for their experiments, cell cultures from Riesling, Solaris and Chardonnay were transferred from the JKI to ScreenSys.

Website:

An email containing the initial idea of a website was circulated in November 2022 and was further discussed at the first project meeting on 10th November. The website in three different languages is **expected to be online by early 2023**. Any further comments and suggestions on the content and organization of the website are most welcome.

Newsletter:

To coordinate the project more efficiently and to keep all the partners informed about the activities, newsletters will be produced and disseminated on regular basis (**once in 3 months**) from KIT-BOT. An email list with all the participating members has already been set up.

Outputs:

Surveys:

Public relation events:

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

- Peter Nick: Interview Campus Radio KIT, 7.11.2022, will go public in January 2023
- Peter Nick: Report in BioPro Baden-Württemberg, 29.11.2022, will go public in January 2023

Talks and seminars:

December 6, 2022, public talk at the Staatliches Museum für Naturkunde, Karlsruhe. Peter Nick "Peter Nick – Wein, Pilz, Klimawandel - Szenen einer Dreiecksbeziehung"

Symposia:

Farmers' awareness:

Scientific achievements:

Latest scientific breakthroughs:

KIT: Mapping heat stress markers in the contrasting pair Riesling (sensitive) and the wild sylvestris Hördt 29 (tolerant) shows that Hördt 29 can sustain transpiration under 42°C, linked with a higher density of stomata. On the molecular level, this is linked with a higher expression of plastidic heat-shock proteins, while Riesling induces cytosolic heat-shock proteins (indicative of perturbed photosynthetic electron transport leading to leakage of reactive oxygen species). The heat tolerance of Hördt 29 is reflected in a high heat tolerance of the Hördt 29 cell line, which tells that it is possible to infer the behaviour of the plant from the behaviour of the individual cells. One of the earliest cellular markers for heat sensitivity versus tolerance is a breakdown of respiratory electron transport detectable by the fluorescent marker MitoTracker Green in the Chardonnay versus the Hördt 29 cell line.

Methods developed:

ScreenSYS: Cultivation, protoplasting and measuring heat-induced mortality are currently established using material transferred from KIT and the JKI.

Both cell lines with contrasting thermotolerance (Chardonnay and Hördt) were transferred to the SCR facility and cultivated. A prerequisite for the quantification of viable protoplasts from cell cultures is the application and read-out of the viability dye fluorescein diacetate (FDA) which gets enzymatically cleaved in viable cells into fluorescent fluorescein. As shown in Figures 1 A and B, cell cultures from both cell lines sampled on day 3 after subculture showed cell clusters with comparable structure and size with most cells fluorescing after FDA application indicating viability as expected. The first approaches to isolate protoplasts according to a procedure applied for the very well-established tobacco cell culture BY-2, however, yielded only a few viable single cells (Figure 1 C; Bandmann et al., 2011, Molecular Plant 2, 241–251). Optimization of digestion medium and enzyme composition will be performed to achieve a number of viable single cells which match those observed before cell wall lysis and which will allow the planned examination of the effect of heat treatment on protoplast viability. Moreover, we observed that after one-day incubation in multi-well plates, isolated protoplasts aggregated in the central well region which would not allow differentiating individual cell responses in the planned experiments (Figure 1D). Therefore, protoplast embedding in the hydrogel matrix will be established as the next step.

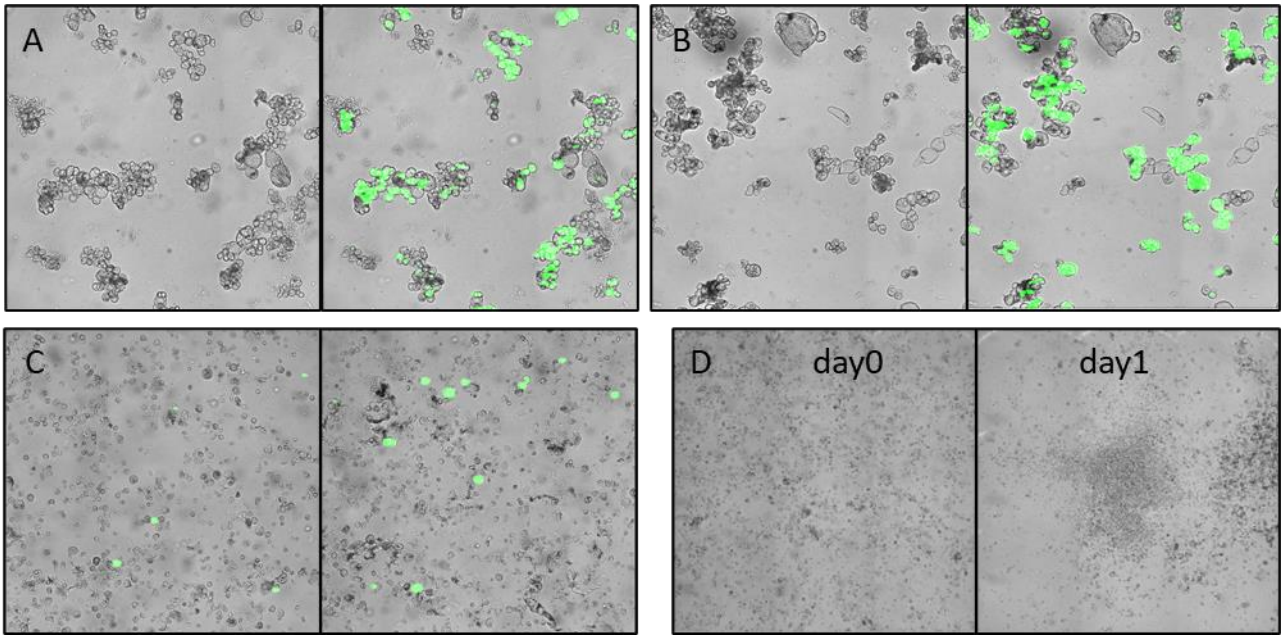

Figure 1. Initial protoplasting approaches *Vitis* cell culture

Cell suspension cultures from Chardonnay (A) and Hördt (B) were incubated with fluorescein diacetate (FDA) as a viability stain. Overlay of fluorescence indicating viable cells on bright field images are shown on the right side each. An initial protoplast isolation did not yield a correspondingly high number of viable protoplasts (C, left: Chardonnay, right: Hördt). Moreover, after one day incubation in liquid medium, protoplasts aggregate in the centre (D, these images show whole well overviews). Improvement of protoplast isolation and embedding in a hydrogel matrix will be the next steps in order to compare protoplast viability upon heat stress.