Project title:
Volatilome of a Cereal Crop-Microbiota System under Drought and Flooding (VolCorn)

Project number: K102/2018

Executive Summary

Extreme weather events increasingly occur through ongoing climate change in Europe. These events, especially droughts and heavy rain events, have caused substantial damage and yield reductions in cereal crop production. A major cereal crop is wheat, and thus was investigated in the project VolCorn. Previous research suggested that the crop microbiota can support the fitness of crops and might be a major target to be harnessed for innovative technologies and cropping systems that require less chemical inputs and are better adapted to climate change, i.e. thus these are more sustainable. We hypothesized that the whole complex of plant host and its colonizing microbiota (bacteria, fungi) respond to abiotic primary stressors and would lead to specific volatile signatures. We demonstrated so far that this is the case. Microbial pathogens enrich under stressors drought and flooding in wheat, and the plant slowdowns its root metabolism and transfers compounds into the above ground part. In combination with herbivores, that we considered as secondary biotic stressor, we detected changes in volatile signatures. For the first time, we combined system biology methods for plant metabolism, microbiota and volatilome analyses to address such complex responses. VolCorn outcomes has fostered our perspective on plants and especially on crops, that we consider as holobionts. The project's outcomes suggested that modern breeding but also crop management should consider cereal crops, such as wheat, as complex metaorganisms that are the target of any management measure (e.g. fertilization, watering regime, pesticide application). This complex interaction of plants with its microbiota bears the potential to increase fitness if plant beneficial microbes are fostered under stress. Routes and measures to achieve this goal can be a major effort to improve cereal crop production.

1. Achievement of objectives and milestones

Extreme weather events increasingly occur through ongoing climate change in Europe. These events, especially droughts and heavy rain events, have caused substantial damage and yield reductions in cereal crop production. A major cereal crop is wheat, and thus was investigated in the project VolCorn. Previous research suggested that the crop microbiota can support the fitness of crops and might be a major target to be harnessed for innovative technologies and cropping systems that require less chemical inputs and are better adapted to climate change, i.e. thus these are more sustainable. We hypothesized that the whole complex of plant host and its colonizing microbiota (bacteria, fungi) respond to abiotic primary stressors and would lead to specific volatile signatures.

The main goal of VolCorn was to understand the response of wheat - as investigated by the variety *Triticum aestivum* L. *var* Chinese Spring. We choose the variety since 2018 an annotated genome – the first of wheat – has been publised. VolCorn aimed for an understanding of the response of the wheat holobiont to the abiotic stresses drought and flooding on the level of (a) plant growth performance and phenologic parameters, (b) phyllospheric and rhizosphere microbiota adaption incl. myccorhiza and (c) its volatilome in one integrative experimental approach. Eventually, we wanted to identify which volatiles may be key to detect the stress state of the holobiont or might be helpful to mitigate the stress consequences, such as decreased plant growth or grain yield.

The partners ZALF (Plant Microbiome, Prof. Dr. S. Kolb), IPK (arbuscular fungi, Prof. Dr. P. Franken, Dr. habil. S. Ruppel), IPK (Plant nutrition and metabolism, Dr. M-R. Hajirhezaei) and iDiv (Chemical Ecology, Prof. Dr. N. van Dam) have worked together To get realistic opportunity to realize this holistic and systemic goal.

VolCorn followed the work program as depicted below (Figure 1). The hired scientific coworkers started in April 2019 and later. This and the restrictions that occurred during the Covid-19 pandemic are the reasons why the project period has been cost-neutrally extended until December 2022.

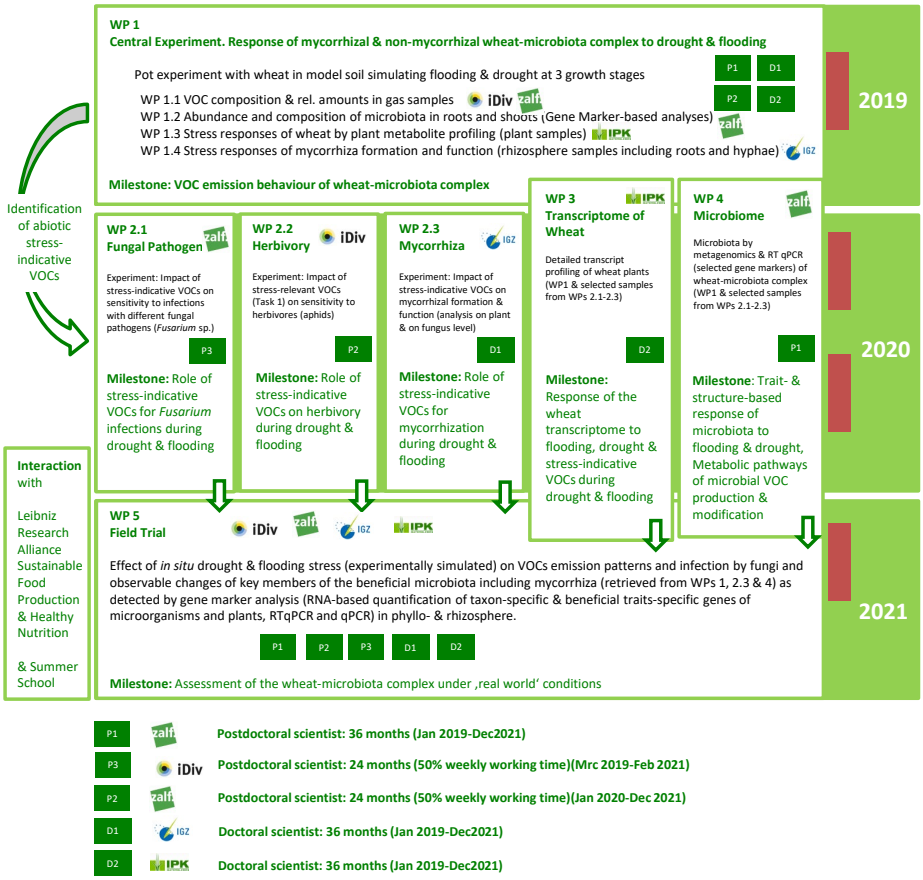

***Figure 1**. Workplan of the Project VolCorn. The formal start of VolCorn was 01.01.2019, newly hired scientific coworkers started 01.04.2019 and later.*

Due to obstacles that occurred caused by lock down measures in some institutes (iDiv, IPK) some miles stones could not been achieved. Such were the planned experiments *Fusarium* infection and interaction with drought and flooding (WP 2.1). These were not feasible since at IPK facilities were locked down. WP2.3. did not work out since a glasshouse required at iDiv was not usable due to regulative issues. Thus, WP 2.2. on herbivory and its interaction with drought and flooding could only worked on a year later. Another issue occurred, since we recognized that the mycorrhization with AMF was not efficient. Likely the chosen variety was not well colonizable. Hence, also WP 2.3 did not reveal results.

2. Activities and obstacles

During the project period (01.01.2019 – 31.12.2022) a physical kickoff (at ZALF, March 2019) and final closing meeting (iDiv, November 2022) were realized. During setting up and conducting the central pot experiment (work package 1, WP 1) a further WP-specific physical meeting occurred at IPK with most project members in 2021. Any further exchange occurred mainly by online meetings or bilatarally through phone and email. Key results from WPs 1and 4 have been presented on the international conference miCROPe 2020 in Vienna, Austria. Further outreach activities did not occur mainly due to severe pandemic restrictive regulations.

Due to measures during the Covid-19 pandemic, the WP 1 associated central pot experiment was repeated after one failure and the retrieved soil, microbiota and plant performance parameter were analyzed and largely published. However, the integration of volatilome data was not possible due to methodological challenges. However, these were solved later in Master theses at iDiv and let to interesting results. A planned experiment with

inoculation with a typical wheta pathogen F- Fusarium spp. – were not conducted due to covid-19 pandemic measures at the partner institute IPK that was responsible for glass house facilities. VolCorn however was well able to assess (a) the response of microbiota (Bacteria, non-mycorrhizal fungi) (WP 4) and (b) were able to resolve the transcriptomic response of wheat host under flooding (WP 3). Moreover, we realized in the last year 2022 a field experiment as proposed in WP 5. However, due to the end of the project the coordinating postdoc Dr. Davide Francioli was hired for a new job. Thus, we are still going forward with further analysis of the data. A publication has not been achieved yet and resources for doing so are beyond the suggest project budget.

3. Results and successes

The major result of our work was that both flooding of the root system but as well drought, affected both the rhizosphere and leaf microbiota in regard to its biodiversity and also its composition as based on taxa identity of Bacteria and fungi. We clearly showed that for both, more pronounced for fungi, microbial groups a shift towards pathogens occurred in both plant compartments (rhizosphere, phyllosphere). This argues for a plant host-driven response to the abiotic stressors, which also shifted the microbiota composition. However, the stressors had strongest effects on wheat plants from early growth stages (e.g. tillering) and less at later growth stages such as flowering or ripening, when obviously the root system and the holobiont has well established. This argues that wheat is under flooding, likely also drought, more susceptible to pathogens at early plant development and that proven for flooding the abiotic stressor fosters this likelihood due to an enrichment of pathogens. A prove of principle would be an experiment in which a typical pathogen would have been added to test the hypothesis, which was not possible due to pandemic restrictions at partner IPK. However, we used together with a further non-funded Partner (Dr. S. Patz, University Tübingen) an new bioinformatic approach to identify in metagenomes genetic traits of the microbiota. We could show (not published, presented in miCROPe conference in 2022) that plant-beneficial bacterial traits have been enriched in rhizosphere. Our non-published results revealed that vitamin B and some plant nutrient-acquisition-associated bacterial traits (iron complexing siderophores, N2 fixation) were statistically significantly enriched in the microbiota of flooding stressed wheat roots.

We could not associate these observations with the volatilome pattern due to methodological issues and described lack of possibilities to conduct alternative experiments in glasshouses. The secondary and biotic stressor herbivory, that we tested with aphid inoculation, definitively shifted the volatilome. This results confirmed observations from other plant hosts. Moreover, we could establish a new data analysis tool (MetIgel) for complex volatilome analysis. An interesting result was that plant-internal resource allocation occurred when the wheat root system was flooded. In this phenomenon the amino acid alanine played a key-role in mediating this phenomenon within the plant. Flooding caused also a slowdown of the overall root metabolism. If this had led to less root exudation and an increase of plant pathogens is likely but requires further investigations. A synthesis of our microbiome and transcriptome data is planned and will help to resolve if key shifts in transcriptional activities of specific pathways in the wheat host was correlated with shifts in microbiota composition, i.e. specific microbial taxa abundance. How these pot-experiment based results can be recovered under real-world conditions (WP5) is currently an open questions but under analysis.

VolCorn was severely hampered by the Covid-19 pandemic. Its cost neutral extension could not solve the occurring challenges. Nonetheless, the project has gained 6 peer-reviewed publications and further one has been revised is under review to date. A professorship of one of the PIs and tenure track professorship for one postdoc were realized. Two doctoral theses are going to be submitted this year (2023) and three Master theses were finished.

Professorships. The coordinator of VolCorn promoted during the project to a joint professor with the Humboldt University and ZALF. The coordination and initiation of the project VolCorn to establish a new research field at ZALF was a substantial part of this achievement.

Postdoc scientists development. One fulltime postdoc scientist has worked as a daily coordinator for the project and published 4 studies at ZALF. This postdoc scientist conducted and coordinated central experiments of WPs 1 and 5, and supported daily coordination of the project consortium. The coordinative postdoc left VolCorn in early 2022 after the position has ran out. He became in *2023 tenure track Professor for Plant Microbiomics at the University Geisenheim*. Another postdoc scientist left after 4 months of work the project due to personal reasons and was substituted by the above mentioned postdoc scientist. This person is now a senior scientist in the private sector. A second postdoc worked at iDiv for 2 years (0.5fte) in VolCorn and established Volatilome analytics incl.

a new data analysis tool MetIgel and (co-)supervised a master thesis candidate at partner iDiv. Thereafter, he acquired a position at the German Biomass Research Centre (DBFZ). **Doctoral coworkers.** During the project one person conducted a phd thesis at partner IGZ and will submit the thesis in November 2023 to Thaer Institute of Humboldt University of Berlin. A second doctoral student conducted a phd thesis at IPK and will submit the thesis in November 2023 to Martin-Luther-University in Halle.

Master Theses. a Master thesis "Effects of interacting biotic and abiotic stress on the wheat volatilome"at Leipzig University in which the voaltilome of the tested wheat variety was investigated(**2022**). Another Master thesis "Der Einfluss volatiler organischer Verbindungen von der Getreideblattlaus befallener Winterweizen-Pflanzen auf benachbarte unbefallene Pflanzen derselben Art." Was achieved at the Ernst Abbe Hochschule, Jena (**2023**). **And third** Master thesis "Frequencies of Microbial Volatile Synthesis and Degradation Genes in the Wheat Rhizosphere Microbiome under Simulated Flooding" was achieved at the Hochschule für nachhaltige Entwicklung Eberswalde (HNEE) (**2022**). That thesis realized a metanalysis on the state of knowledge on microbial volatiles and their effecte on plant hosts under abiotic stressors.

Peer reviewed publications from VolCorn:

1. Pauwels, R., Jansa, J., Püschel, D., Müller, A., Graefe, J., Kolb, S., & Bitterlich, M. (**2020**). Root growth and presence of Rhizophagus irregularis distinctly alter substrate hydraulic properties in a model system with Medicago truncatula. *Plant and Soil*, *457*(1-2), 131-151.
2. Francioli, D., Cid, G., Kanukollu, S., Ulrich, A., Hajirezaei, M. R., & Kolb, S. (**2021**). Flooding causes dramatic compositional shifts and depletion of putative beneficial bacteria on the spring wheat microbiota. *Frontiers in Microbiology*, *12*, 773116.
3. Francioli, D., Lentendu, G., Lewin, S., Kolb, S. (**2021**) DNA metabarcoding for the characterization of terrestrial microbiota - pitfalls and solutions. Microorganisms 9, 2, Article 361.
4. Francioli, D., Cid, G., Hajirezaei, M.-R., Kolb, S. (**2022**) Leaf bacterial microbiota response to flooding is controlled by plant phenology in wheat (*Triticum aestivum* L.). *Scientific Reports* 12, 1, Article 11197.
5. Francioli D, Cid G, Hajirezaei MR, Kolb S. (**2022**) Response of the wheat mycobiota to flooding revealed substantial shifts towards plant pathogens. *Frontiers in Plant Science* 13:1028153.
6. Pauwels, R., Graefe, J., & Bitterlich, M. (**2023**). An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. *Mycorrhiza*, *33*(3), 165-179.

Presentation of Results on International Conferences

The international conference **miCROPe** (**https://www.micrope.org/**) is focused on crop microbiome research and occurs every 2 years since 2017. It joins scientific participants but as well stakeholders from biotech industry. It is also joined with a European crop breeding workshop. Participants (about 300) are high level scientists (microbiome and crop plant scintists incl. breeding experts) from more than 30 countries from the whole world. This conference gave the research topic and the project VolCorn high visibility and has led to further collaborations and scientific activities at ZALF.

- **Talk/oral presentation** – miCROPe 2019, held in Vienna 2022: Wheat mycobiota responses to flooding revealed substantial shifts towards harmful fungi Davide Francioli, Geeisy Cid, Sonja Wende, Mohammad-Reza Hajirezaei and Steffen Kolb
- **Talk/oral presentation** – miCROPe 2019, held in Vienna 2022: Functional Traits of Wheat's Bacterial Rhizosphere Microbiota under Flooding and Drought by Metagenomics. Sonja Wende, Sascha Patz, Silke Ruppel, Steffen Kolb

Publication under revision (29.09.2023):

- Geeisy Cid, Davide Francioli, Steffen Kolb, Yudelsy Antonia Tandron Moya, Nicolaus von Wirén, Mohammad-Reza Hajirezaei (**2023**) Elucidating the systemic response of wheat plants under waterlogging based on transcriptomic and metabolic approaches. bioRxiv 2022.08.03.502608 / UNDER Revision Journal of Experimental Botany.

Data Analysis Tool (R-based)

- Andreas Schedl &Linnea Smith. Freely available R-based tool to create sunburst plots of volatile and non-volatile metabolomic data – MetIgel; https://codeberg.org/MetIgel/MetIgel/src/branch/MetIgel_v1.0

4. Equal opportunities, career development and internationalisation

All partners were committed to support the hiring of female scientists. A coordinative postdoc who left the project due to personal reasons was female. Two out three MSc students were female and one of the two doctoral students was female too. Most of the hired coworkers had an international background. In all partner institutions English was all day language and all meetings were held in English. In one case, a postdoc could further develop in regard to a career step (Professorship) in academia and a second was able to get permanent position in the biotech industry. Moreover, we think that both doctoral students will promote in 2024 at the universities Martin-Luter University Halle and Humboldt University Berlin. Major outcomes of VolCorn were presented on an international and globally well visible conference in 2022 (miCROPe, Vienna, Austria).

5. Structures and collaboration

The specific experiments were done with personnel hired at the partner institutions. Despite Covid-19 pandemic 2019, 2021 and 2022 meetings with all partners and coworkers were held physically. The project coordinator supported by the coordinating postdoc scientist ensured a regular exchange on important organisational issues, especially for central pot experiment of WP1 and the central field experiment of WP5. Originally, more regular and physical meetings were planned, i.e. to foster team building, which was not possible due to restrictive regulations during the Covid-19 pandemic. However, for both central experiments (section 1) also all partner institutions supported the conductance at IPK (Pot experiment, WP1) and ZALF (Field experiment, WP5) by sending personnel.

6. Quality assurance

All partners were informed and familiar with rules of Good Scientific Practice as published by the Deutsche Forschungsgemeinschaft. All involved partner institutes, i.e. Leibinzzentrum für Agrarlandschaftsforschung (ZALF), Deutsches Zentrum für integrative Biodiversitätsforschung (iDiv), Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung Gatersleben (IPK), and Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZ) committed themselves by institutional policies to follow these and delineated rules of the Leibniz Gemeinschaft. Moreover, all involved PIs have been regular fundings from DFG and made sure due to personal communication with the hired coworkers that also these persons are informed about it. Quality was also ensured due to individual supervision of the main authors through PIs during writing and regular exchange on data and methodologies between partners and co-authors.

7. Additional resources

The challenges that occurred during the project made it necessary to support the experimental work with additional household personnel (technicians) and gardeners at IPK and ZALF. Also additional scientist worktime at iDiv was afforded to get the project goals done. Of course, all partner institutes provided devices and glass house infrastructure that were financed *in kind*. Especially, DNA sequencing cost of additional 19,000€ were provided by ZALF to realize scientific goals. We did not monitor the costs, but without substantial *in kind* financial support for consumables and also additional personnel in all four partner institutions presented and planned results would not have been possible, since experimental works requires a high share of non-personnel costs.

8. Outlook

Our work led us to the following open questions: (1) Is the impact of flooding or drought increasing the susceptibility of the wheat to be infected by typical pests? (2) Is the wheat holobiont volatilome informative for health state of the wheat holobiont? (3) Is the resource allocation in the wheat host under abiotic stress

mechanistically linked with enrichment of plant-beneficial rhizosphere microbes? We will follow these questions by not finished data analysis and publication. For ZALF, this research was an opportunity to develop a new line of research that will be further established in the upcoming years.

Appendices

A1. Performance Report (1 page)

A2.-A5

These appendices are the individual self-reports of the partners that give much more detailed information on the scientific outcomes of VolCorn. These appendices are only for Leibniz-internal usage and not for general public, since not all results have been fully evaluated and published at the date of the submission of the final report (29.09.2023).

Coordinator (Prof. S. Kolb) of VolCorn, ZALF, Müncheberg
29.09.2023