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D22.1: Summary of access arrangements for UCPH

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Executive Summary

Objectives
PhenoLab is a fully automated, high-throughput phenotyping platform comprising of an automatic system for plant care and movement in combination with a vision station for monitoring and measurements. Special features: (1) High sensitivity, multispectral imaging systems with discrimination of the wavelengths by narrow banded LEDs for enhanced sensitivity (2) Multifluorescence imaging in combination with 10 different light sources allow a large number of combinations of excitation and emission wavelengths (3) Individual measurement of soil water content in the pots and watering based on the measured values (4) Thermo-imaging inside the imaging box and also outside with cultivation light condition(5) Flexible change of the orientation and position of pots for randomization. (6) Physiological phenotyping by enzyme activity signatures and phytohormone profiles.

Main Results:
Number of accesses: 7
Persons trained: 7
Key outcomes and benefits:
- for users: combination of automated sensor based and cell physiological phenotyping
- for installation: testing of additional plant species and pathogens and new combination of treatments
- for both: establishing new collaborations

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1. Overview of TNA users projects realized in TNA UCPH

1.1.1. InstallationS (short description of each installation in the local infra)

PhenoLab is a fully automated, high-throughput phenotyping platform comprising of an automatic system for plant care and movement in combination with a vision station for monitoring and measurements. The automatic system consists of 13 lanes and a circular transport lane of conveyor belts with space for up to 117 fixtures. One plant (13x13 cm pot) or four plants (8x8 cm pots) can be mounted in each fixture. The fixtures can be randomized, rotated, photo measured, weighed and irrigated up to twice a day. Irrigation with up to four different nutrient mixtures can be done either at the soil level or into the fixture reservoir. A very special feature is that the soil water potential is measured continuously by individual sensors in each fixture and the individual irrigation amount is adjusted according to the measured water content of the soil, thus this system is particular suited for defined drought experiment. The maximum plant size in PhenoLab is 60 cm height and 25 cm width. The vision station consists of a box with excluded day light where cameras and LEDs are mounted. Before entrance to the vision box the plants height is measured to adjust the camera position.

The vision station is equipped with 2 thermo cameras (LWIR 8000-15000 nm) - one inside and one outside the box which allows to assess the temperature control also in the presence of cultivation light conditions. A high sensitivity, multispectral imaging systems (VISLab) is mounted inside the box with the special feature of the discrimination of the 10 wavelengths by narrow banded LEDs for enhanced sensitivity within the in range from 365 to 970 nm. In addition, the system provides the possibility to perform multifluorescence imaging, while the combination of the various light sources with 6 fluorescence filters allow a large number of combinations of excitation and emission wavelengths. Instrumentation is available for complementary analyses of specific physiological source specific parameters via non-invasive techniques, including gas exchange and PAM fluroescence imaging. As a special service the established analytical platforms for the determination of activity signatures of 13 key enzymes of carbohydrate metabolism and profiles for 16 phytohormones will provide the analyses for a subset of up to 40 samples.

1.1.2. User projects

Min. quantity of access units to be provided according to the DoA: 6
Total number of access units (sum of access units in the table): 7

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 ?
PhenoLab	Assessing the cost of resistance in potato	ResPot	Assessemt of growth	Erik Alexandersson	Yes	experiment	1	1 paper submitted	2
PhenoLab	Wheat phenotyping for a warmer and drier climate	HeatDroughtPheno	Asesment of drought and heat responses	Pedro Correia	Yes	experiment	1	1 paper planned	1
PhenoLab	Terminal drought and heat-effects on chickpea flowering, seed yield and quality	FloweringUnderStress	Assessment of drought and heat response	Carla Pinheiro	Yes	experiment	1	1 paper planned	2

PhenoLab	Analyses of the influence of leaf rust (Puccinia triticina Erikss.) on carbohydrate and antioxidant metabolism and phytohormones of wheat by combining multispectral imaging with physiological phenotyping	PhenoLeafRust	Assessmet of pathogen response	Valentina Spanic	Yes	experiment	1	1 paper planned	1
PhenoLab	Physiological phenotyping under controlled conditions of durum wheat accessions differing for heat stress tolerance in field conditions	PHYSIO-DURUM-HEAT	Assessment of drought response	Roberto Tuberosa	Yes	experiment	1	1 paper planned	1
PhenoLab	Exploration of historical breeding progress in Norwegian spring wheat by combining high throughput sensor based and physiological phenotyping methodologies	HisNordic	Assessment of growth	Tomasz Mroz	Yes	experiment	1	1 paper planned	0
PhenoLab	Assessing microalgae derived properties through plant phenotyiping	MiBiP	Assessment of growth	Eleonora Sforza	Yes	experiment	1	1 paper planned	0

** 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

ResPot, Assessing the cost of resistance in potato
Plants have evolved an array of defenses against pathogens. However, mounting a defense response comes with a certain fitness cost in the form reduced growth and reproduction. This has implications in both natural and agricultural populations, where it in the former this fitness cost can be a driver of local adaptation whereas it in the latter it can reduce yield and consequently affect food production. Here we want to measure this cost with advanced phenotyping. In this experimental set-up, we want to use the PhenoLab at University of Copenhagen to measure the growth rate, architecture and changes in development (phenology) as well as the effect on photosynthesis and plant health of plants treated with plant resistance inducers (PRIs) and transgenic potato plants carrying additional resistance genes. In addition, we want to study a Coi1 mutant in potato, which is JA insensitive, and thus is impaired in mounting parts of the stress defence. In field conditions, this has an early senescence phenotype. From this experimental set-up we expect to see differences in growth dynamics, shoot biomass, height, size morphology as well as photosynthetic status (quantum yield, non-photochemical quenching), stress response and leaf health status after PRIs treatment or as an effect of additional R-genes. These measurements will be possible to relate to already obtained data from global gene expression studies as well as to yield from field studies and other phenotypes observed in the field.
Persons trained: 2

HeatDroughtPheno, Wheat phenotyping for a warmer and drier climate
Around 40% of the global wheat yield fluctuations are explained by climatic variation, and heatwaves and drought are among the principal stressors. In this project ten wheat genotypes, characterised during the SATYN experiment (Stress Adaptive Trait Yield Nursery, CIMMYT), were evaluated under high temperatures (38 /31 ℃, day/night) and different watering regimes. Most of these lines were characterized as the top performers during SATYN in dry or warm environments, and additionally low performers for drought and heat stress were added to the experimental design. The aim of this high-throughput phenotyping experiment was to study how stomatal regulation (gs) and water use efficiency affects carbon allocation in genotypes adapted to different environmental conditions. Thermography was used to estimate gs and transpiration, and multispectral and RGB imaging were applied to track changes in morphophysiological parameters of wheat genotypes exposed to high temperatures and/or drought. At the end of the experiment leaf samples were collected, snap frozen in liquid nitrogen and stored at -80 ℃. In a second step, a semi-high-throughput protocol was performed to quantify the activity signatures of key enzymes of carbohydrate metabolism. Data from plant growth sensing and metabolites flows are being integrated and helping to understand how stomatal regulation and carbon allocation can determine wheat productivity in warmer and drier climates.
Persons trained: 1

FloweringUnderStress, Terminal drought and heat-effects on chickpea flowering, seed yield and quality
Chickpeas (Cicer arietinum L.) are included in the Zero Hunger program, an initiative of World Food Programme for food safety and better nutrition. Chickpea is the world's second most cultivated food legume, and first in the Mediterranean basin, and has one of the best nutritional compositions among the dry edible grain legumes (Kashiwagi et al. 2013; Ribeiro et al. 2017). Drought typically reduces chickpea grain yield, namely when occurring during pod set and seed filling (Duarte-Maçãs 2003; Krishnamurthy et al. 2010; Pang et al. 2017). Reported yield losses due to drought vary between 36 and 42% (Saxena et al. 1993; Khodadadi 2013) and in

combination with elevated temperatures seed filling is further compromised and yield further reduced (Devasirvatham et al. 2015). In this project, two chickpea genotypes developed under the Portuguese Breeding Program at Portugal (Elvas, INIAV) were evaluated regarding terminal drought in combination with high temperatures (performed at Phenolab). Elvar was the 1st cultivar developed and is available from the Portuguese varieties catalogue since 1993. Adapted to dry conditions, it as a high production potential (100 seed weight ranging from 32-38 g) and is the main variety produced in Portugal and South of France. CHK5810 was in the meantime released as Electra (2020) and was selected due to its production potential (100 seed weight ranging from 45-48 g) and large seed size. We focused the phenotyping during flowering and seed filling stages, the most critical stages for seed yield. The goal is to evaluate the physiological responses at plant level relating it to the activity signatures of key enzymes of carbohydrate and antioxidant metabolism. We target both the leaves and the seeds. Our experimental design included the analysis of plants from each genotype that were kept in the greenhouse to serve as a normalising control (to account for the impact of the pot effect). The phenotyping approach at Phenolab will be complemented with seed production (yield) and quality analysis. One additional goal is to compare the seeds obtained at Taastrup (Danmark) with the seeds obtained at Elvas (Portugal). With this project, we aim to contribute to understanding chickpea productivity under warmer and drier climates by gathering information on how sink capacity is modulated by challenging environmental conditions (single and/or combined).
Persons trained: 2

PhenoLeafRust, Analyses of the influence of leaf rust (Puccinia triticina Erikss.) on carbohydrate and antioxidant metabolism and phytohormones of wheat by combining multispectral imaging with physiological phenotyping

Wheat may be attacked by a large number of diseases and pests, but some have global distribution, such as leaf rust (Puccinia triticina Erikss.). Leaf rust, has a great economic impact on the cereal industry due to reduction in the yield and end-use quality of wheat genotypes. Each year, across Europe, severe epidemics of wheat leaf rust in combination with yellow rust occurred and there was a significant yield loss in susceptible wheat genotypes, when rusts were responsible for 40-50% yield loss. Epidemic conditions of leaf rust can occur early in the growth season on the leaves or later on the spikes (high humidity and temperatures 15°C to maximum 23°C) which can lead to severe outbreaks. The best approach to control leaf rust is to use the least susceptible wheat varieties with effective resistance genes which is the most environmentally and economically efficient approach. Under pathogen infestation, plants activate numerous specific mechanisms that partially restrict pathogen extracellular and intracellular growth and penetration. In the early stage of plant-pathogen interaction, plant produces excessive concentration of reactive oxygen species (ROS), like H2O2 , superoxide (O2 .-) and hydroxyl (OH.) radicals, that can cause irreversible changes in the cell like unspecific oxidation of proteins and/or nucleic acids and membrane lipids degradation resulting in loss of physiological functions. The purpose of this project is to find the wheat genotypes that have a certain level of resistance to leaf rust infections by linking physiological and the optical scales in a high-throughput approach using multispectral imaging at the early stage of infection. In general, we want to check with high-throughput technique with controlled environment if this will lead to effective and efficient screening of wheat-leaf rust interactions. Multispectral imaging is a powerful non-destructive tool for detection of early disease changes in chlorophyll fluorescence which should be quite interesting in the point of photosynthesis view which plays an important role in modern winter wheat varieties. In addition, the activity of antioxidant metabolism enzymes (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, glutathione, S-transferase and apoplastic peroxidase) with kinetic enzyme activity assays, antioxidant potential and compounds, and lipid peroxidation in a 96-well microtiter plate was measured. There are not many studies on comparing of antioxidative enzymes in susceptible and resistant wheat varieties upon the inoculation with leaf rust, so therefore

multispectral imaging and the activity of antioxidant metabolism enzymes are of great value for this research.
Persons trained: 1

PHYSIO-DURUM-HEAT, Physiological phenotyping under controlled conditions of durum wheat accessions differing for heat stress tolerance in field conditions
This study focused on drought stress rather than heat stress as originally planned, because the 2-month grant would not have allowed to complete two phenotyping cycles at two different temperatures with plants reaching maturity. Additionally, investigating drought effects at the biochemical level allows for a more complete characterization of the 16 genotypes already tested under well-watered (WW) and water-stressed (WS) regimes in the field at Maricopa and in the greenhouse at IPK. This experiment investigated how osmotic adjustment (OA) regulation affects leaf relative water content (RWC), water use, water-use efficiency, drought tolerance and the activity of key enzymes of carbohydrate metabolism. Multispectral and RGB imaging were applied to track changes in morpho-physiological parameters of the 16 genotypes exposed to WW and WS conditions. Leaf samples were collected, snap-frozen and stored at -80℃. A second step, still ongoing, evaluated the activity of key enzymes of carbohydrate metabolism. Project activities 1) To evaluate under WW and WS conditions in the PhenoLab platform 16 durum wheat accessions contrasted for OA capacity as previously evaluated under progressively increasing in Maricopa (USA). 2) To determine (a) activity of key enzymes of primary carbohydrate metabolism (resource/assimilate allocation), (b) antioxidant metabolism, (c) phytohormone profiles (regulation) in drought stressed plants.
We characterized for water use and water-use efficiency 16 durum wheat genotypes differing in OA capacity under drought conditions. Dissemination and publication Data is being collected as part of a Master's Degree project. A manuscript will be submitted in 2022 to a peer-reviewed journal.
Persons trained: 1

HisNordic, Exploration of historical breeding progress in Norwegian spring wheat by combining high throughput sensor based and physiological phenotyping methodologies
Breeding progress is achieved by crossing cultivars and breeding lines with complementary traits, followed by screening the offspring for individuals showing promising phenotypes. This phenotypically driven method can be considered somewhat a "black box" – most often the progress is achieved without knowing its exact physiological or genetic basis. A deeper insight in it is needed on both scientific and practical planes: in the applied dimension, it can be determined which of the traits the progress had relied on and which features still show room for future improvement (phenomics), which genetic structures were associated with the advances (genomics) and how the physiological architecture is arranged and related to the breeding outcome. Such knowledge, especially when combined, can provide directions for future breeding programs by enabling direct selection at different omics level, thereby accelerating genetic gains, and reducing the required workload. The project aimed to determine if the historic yield increase in Norwegian spring wheat (T. aestivum L.) can be explained at physiological and metabolomic levels through combining novel HTP (high-throughput phenotyping) and semi-HTP biochemical methodologies offered at PhenoLab, University of Copenhagen. The main goal was to study the differences in crop canopy development, photochemical performance and multispectral patterns supplemented with cell physiolological (metabolomic/phytohormone) signatures over the vegetation period in a set of spring wheat cultivars representing 5 decades of breeding progress in Norway. Results provide multi-level knowledge of the basis of the yield progress: on cell physiological level (enzymatic signatures, phytohormones) and the whole plant level (multispectral) as well as high-dimensional data on temporal dynamics of growth and development at the single plant level. The project was carried out as planned with some changes due to the pandemic, which did not affect the outcome. Data and samples gathered in the project are being analysed and

integrated to provide a multi-level understanding of the progress present over the last five decades in Norwegian spring wheat breeding.
Persons trained: 0 (due to Covid-19 restrictions)

MiBiP, Assessing microalgae derived properties through plant phenotyiping
The increasing world population and the effects of climate changes and global warming are a current and strong concern for the future. To ensure food availability and security, the development of efficient and sustainable agriculture practises is required and biostimulants represent a possible approach to improve crops yield reducing plant stress. In recent years, microalgae arouse interest for their application as plant biostimulants for a wide variety of crops since they are able to produce many bioactive compounds possibly involved in biostimulation. The aim of this project was to investigate the effects of four different microalgae species on tomato plants (L. esculentum L.), using a phenotyping approach to evaluate possible growth improvements that might be species-specific. Microalgae were cultivated at the University of Padova and the harvested biomass was subjected to enzymatic hydrolysis at SICIT Group S.p.A. before performing the bioassays. Different microalgae doses and formulations were applied to seedlings during the experimental campaign and their growth was monitored daily, in order to assess the dose-response effects and possible outcome of the plant development. Enzyme signatures was analysed in order to assess microalgae effect at molecular level. Finally, further experiments were carried out with two microalgae species on tomato plants cultivated in big pots, in order to investigate the effect of microalgae treatments until fructification stage. Thanks to the experiments carried out, it is possible to assess the actual advantage of using microalgae as biostimulant, addressing the main open questions, which include the suitable dosages, the stage of the plants to be treated and the time interval among subsequent treatment applications.
Persons trained: 0 (due to Covid-19 restrictions)

2.1.2. Selection of One exemplary project

HeatDroughtPheno, Wheat phenotyping for a warmer and drier climate
Interannual and local fluctuations in wheat crop yield are majorly explained by abiotic constraints, being heatwaves and drought among the top stressors. Moreover, these stresses commonly co-occur, and their frequency is increasing with global climate change. This work optimized high-throughput methods to phenotype wheat plants under controlled water deficit and high temperature, with the aim to identify the phenotypic traits conferring adaptative stress responses. Ten wheat genotypes were characterized and classified on their water use and growth dynamics, to ultimately understand the regulatory mechanisms on the primary carbohydrate and antioxidant metabolisms under these stress conditions. Wheat plants were grown in a fully automated plant facility under 25/18ºC day/night for 23 days, and then the temperature was increased for seven days (38/31ºC day/night) while maintaining half of the plants well irrigated and half at 30% field capacity. Thermal and multispectral images and pot weights were registered twice daily. At the end of the experiment, key metabolites and enzyme activities from the carbohydrate and antioxidant metabolisms were quantified. Regression machine learning models were successfully established to predict plant biomass by image-extracted parameters. Evapotranspiration traits expressed significant genotype-environment interactions (GxE) when acclimatization to stress was continuously monitored. Consequently, transpiration efficiency was identified as essential to maintain the balance between water-saving strategies and biomass production in wheat genotypes growing under water deficit and high temperature. Tolerance to these stresses included changes in the carbohydrate metabolism, particularly in the sucrolytic (cytINV) and the glycolytic pathways (HXK, PFK), and in the antioxidant metabolism (POX and phenolic compounds). The observed genetic differences in sensitivity to high temperature and water deficit can be exploited in breeding programs to improve wheat resilience to climate change.

3. Reflection on results of the TNA programme

The project provided the chance to test the installation with additional plant and pathogen species in combination with novel combination of factors. The project also contributed to advance and optimize administrative organisational procedures.
The project generated also the possibility for novel collaboration and contributed to attract attention to potential new users.