

D18.1: Summary of access arrangements for FZJ

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This project has received funding from the European Union's Horizon 2020 research and innovation programme rant agreement No 731013. This publication reflects only the view of the author, and the European Commission is held responsible for any use which may be made of the information contained therein.

Document information

EU Project N°	731013	Acronym	EPPN ²⁰²⁰		
Full Title	European Plant Phenotyping Network 2020				
Project website	www.eppn2020.plant-phenotyping.eu				

Deliverable	N°	D18.1	Title	Summary of access arrangements for FZJ					
Work Package	N°	WP18	Title	TA12 - Transnational access to the Infrastructure FZJ					

Date of delivery	Contractu	al 31/10/2021	Actual	12/11/2021		
		(Month 54)		(Month 55)		
Dissemination level	X PU P	ublic, fully open, e.g. web				
	CO Confidential, restricted under conditions set out Grant Agreement					
	CI Cla Decis	as referred to in	Commission			

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Version log			
Issue Date	Revision N°	Author	Change
03/11/2021	1	Simone Gatzke	First version
		Roland Pieruschka	
09/11/2021	2	François Tardieu	Reviewed by
		_	Coordinator





Executive Summary

Objectives

The Installations GrowScreenChamber, GrowScreenRhizo, RootScreen-MRI, are located at the Forschungszentrum Jülich, Institute for Bio- and Geosciences (IBG), IBG2: Plant Sciences and BreedFACE is located at the Campus Kleinaltendorf.

- GrowscreenChamber consists of two growth chambers for routine evaluation of Arabidopsis and other rosette plants. The environmental conditions, temperature, humidity, light intensity and quality, [CO2] are controlled allowing flexible environmental conditions.

- GrowScreenRhizo allows phenotpying of root and shoot growth and architecture of crop plant grown in soil-filled rhizoboxes.

- RootScreen-MRI is a unique system fully dedicated to measure dynamic changes in structural and functional traits of whole root architecture *in situ* in 3D

- BreedFACE, Free Air CO2 Enrichment (FACE) installation to investigate the response of crops to elevated CO2 under field conditions is an octagon of laser drilled pipes which supply CO2 by a pc controlled system.

Main Results

- GrowscreenChamber:

The demand for the use of phenotyping platforms fully dedicated to the model plant Arabidopsis remains high and as such, the access was very competitive. In total 4 experiments were performed to investigate the response Arabidopsis to drought (2 experiments), heat stress (1 experiment), epigenetic landscape of different populations (1 experiment), 4 users were involved in supporting the experiment (training) and 4 publications are expected.

- GrowScreenRhizo:

The facility provides a large flexibility in terms of investigating root properties under different environmental conditions. As such a diversity of scientific questions were address and new crops such as sunflower were used in TNA to establish protocols for different stresses, and to adapt the system to simulate field conditions. Thus, access to GrowScreenRhizo provided an important for high-level publication for example on gravitropism (Kirschner et al 2021 PNAS). In total 6 experiments were performed to investigate the root response to drought (2 experiments) and salt stress (1 experiment), gravitropism (1 experiment), toxicity tolerance towards natural herbicides (1 experiment), root image segmentation software development (1 experiment). 9 users were involved in supporting the experiment (training) and 6 publications are expected.

- PlantMRI:

The PlantMRI facility is unique for the measurements of 3D root dynamics *in situ* in soil. In total 3 experiments were performed to investigate the root response to drought (2 experiments) and to soil compaction (1 experiment). 4 users were involved in supporting the experiment (training) and 4 publications are expected.

- BreedFACE:

The Facility allows experiments under elevated CO2 in the field and was as such one of two facilities in the field with semi controlled environmental conditions. The demand for access was high indicating the need for field phenotyping. In total 4 experiments were performed to investigate the response legumes nutrition under elevated CO2 (1 experiments), physiology and yield of wheat cultivars accounting for 60 years of northern-European breeding (2 experiment) and potato blight resistance under elevated CO2 (1 experiment). 6 users were involved in supporting the experiment (training) and 4 publications are expected.

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Table of contents

Document in	nformation	2
Executive Su	ummary	3
Table of con	itents	5
1. Ove	erview of TNA users projects realized in TNA FZJ (IBG2)	6
1.1.1.	Installations (short description of each installation in the local infra)	6
1.1.2.	GrowScreenRhizo	6
1.1.3.	Plant-MRI	6
1.1.4.	BreedFACE	6
1.1.5.	GrowscreenChamber	6
2. TN	A projects summary	7
3. TN	A projects description	9
3.1.1.	GrowScreenRhizo	9
3.1.2.	PlantMRI	12
3.1.3.	BreedFACE	16
3.1.4.	GrowScreenChamber	19
4. Ref	flection on results of the TNA programme	21
4.1.1.	GrowScreenRhizo	21
4.1.2.	PlantMRI	21
4.1.3.	BreedFACE	22
4.1.4.	GrowScreenChamber	22
5. Ref	ferences	22





1. Overview of TNA users projects realized in TNA FZJ (IBG2)

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

1.1.2. GrowScreenRhizo

The installation allows phenotpying of root and shoot growth and architecture of crop plant grown in soil-filled rhizoboxes (Nagel et al. 2012). The system consists of two rows of mounting frames with rhizoboxes (90x70x5 cm) with a transparent plate for non-invasive root phenotyping. A cabinet for imaging is moving automatically in which images of the shoot and roots are acquired with up to 170 plants per hour. A number of root traits such as root length, spatial distribution of roots including root length density, rooting depth well as shoot traits are analyzed routinely. Annually about 8 experiments are performed, on average 4-5 of them with international scientists resulting in a number of publications (Gioia et al 2015; Avramova et al 2016).

1.1.3. Plant-MRI

The installation is a unique system fully dedicated to measure dynamic changes in structural and functional traits of whole root architecture in 3D. The method yields information on root traits: mass, length, diameter, tip number, branching angles and spatial distribution (van Dusschoten et al., 2016). Plants are grown in tubes of 8.1 or 11.7 cm inner diameter and 30-40 cm height handled by a robot. The capacity of automated measurement is ca. 20 plants per day (scan time 20 minutes) and can be repeatedly measured for the respective scientific question (e.g. every day or week) on diverse root systems. The system offers about 20 experiments per year and on average 10 experiments include international scientists (Schmittgen et al 2015, Metzner et al 2014 and 2015).

1.1.4. BreedFACE

Free Air CO2 Enrichment (FACE) installation to investigate the response of crops to elevated CO2 under field conditions is an octagon of laser drilled pipes which supply CO2 by a pc controlled system. CO2 released from the pipes results in a constant [CO2] level of 550-590 ppm over the vegetation and is supplied for the full growing season. 3 octagonals of 18 meter in diameter allow for 252 plots of 1.5x1.5 under elevated CO2 and a multitude of area is reserved for growth at ambient [CO2]. The system will be tested in 2016 and first experiments are planned in early 2017.

1.1.5. GrowscreenChamber

Installation consists of two growth chambers for routine evaluation of Arabidopsis and other rosette plants. The environmental conditions, temperature, humidity, light intensity and quality, [CO2] are controlled allowing flexible environmental conditions. The workflows are fully automated with stations for the measurement of leaf growth, morphometric parameters, PSII chlorophyll fluorescence, and 2D plant geometric features. The capacity depends on the size of the plants ranging between 400 and 3000. Annually about 10 experiments are performed, 5-6 of them with international researchers (Barboza-Barquero et al 2015; Caliandro et al 2013; Nunes C et al 2012).





2. TNA projects summary

Installation	Min. quantity of access units to be provided according to the DoA	Total number of access units (sum of access units in the table):
GrowSceen- Rhizo	120 (days)	393
Plant MRI	80 (days)	83
BreedFACE	1 (experiment)	4
GrowScreenCha mber	100 (days)	182

Installation	Project title	Project acronym	Coordinator	Already used installation	Unit of access (real)	Number of used access units during the project	(Potential) paper	How many people were trained by this procedure?
	Utilisation of automated phenotyping							
GrowSceen-	platform in examination of water		Dragana					
Rhizo	sensitivity in sunflower – going deep	SunDeep	Miladinovic	No	days	89 days	1	1
GrowSceen-	Characterization of Wheat gEnetic Resources with high high thrOughput rOot phenotyping to increase wheaT							
Rhizo	adaptation to drought	WEROOT	Michela Janni	No	days	72 days	1	1
GrowSceen-	Testing root hypergravitropism as a trait							
Rhizo	affecting drought tolerance in barley	HYPEROOTS	Silvio Salvi	No	days	96 days	2	2
GrowSceen- Rhizo	Development of a fully automated root image segmentation software	RootPhenAlgo	Bastien Billiot	No	days	5 days	0	1
GrowSceen- Rhizo	Characterising autotoxicity tolerance in crops releasing natural herbicides.	RHIZOTOX	Claude Becker	No	days	51 days	1	2
GrowSceen- Rhizo	High throughput phenotyping of durum wheat accessions under salt stress	SAPHE	Mario Augusto Pagnotta	No	days	80 days	1	2
Plant-MRI	Isohydry of potato plants and tuber water capacitance	PoTuWa	Katharina Huntenburg	No	days	51 days	1	1
Plant-MRI	Root Dynamics Under Varying Water Supply	RooDy	Samuele Ceolin	No	days	24 days	1	1





	How does root systems adapt to pore							
	networks in compacted soils? – Impacts							
	on root system architecture and energy							
Plant-MRI	cost of barley	Root-to-Pore	Andong Shi	Yes	13 Days	8 days	1	1
	Effects of FUTURE atmospheric elevated							
	CO2 on LEGUME nutrition, growth and		Marta					
BreedFACE	molecular profiling	FUTURE-LEGUME	Vasconcelos	No	experiment	1	1	2
	The influence of elevated atmospheric							
	[CO2] on physiology and yield of wheat							
	cultivars accounting for 60 years of							
BreedFACE	northern-European breeding	HYSTORYCO2	Tracy Lawson	No	experiment	1	1	1
	Influence of elevated CO2 concentration							
	on potato plants, late blight resistance,							
	and effectiveness of plant resistance		Murilo Araujo					
BreedFACE	inducers (PRIs)	PRIFACE	Sandroni	No	experiment	1	1	2
	The Influence of Elevated [CO2] on							
	physiology and yield of wheat cultivars							
	accounting for 60 years of northern-							
BreedFACE	European breeding.	HYSTORYCO2_II	Tracy Lawson	Yes	experiment	1	1	1
	Studying the Epigenetic Landscape of							
	Different Populations of Arabidopsis							
GrowScreenCha	thaliana through their phenotypic	Phenotypic						
mber	variations	Variation	Ueli Grossniklaus	No	days	60 days	1	1
GrowScreenCha	Characterisation of splicome function in							
mber	Arabidopsis tolerance to water stress	OASES	Rafael Catalá	No	days	44 days	1	1
GrowScreenCha								
mber	The role of PLC in drought tolerance	PLC-Drought	Teun Munnik	No	days	45 days	1	1
GrowScreenCha	Phenotyping of BPM1-mediated heat		Željka Vidaković-					
mber	stress response in Arabidopsis thaliana	PhenoArHeat	Cifrek	No	days	33 days	1	1





3. TNA projects description

3.1.1. GrowScreenRhizo

• SunDeep: Utilisation of automated phenotyping platform in examination of water sensitivity in sunflower – going deep

Within the project we explored mechanisms underlying drought tolerance by analysing sunflower root system. Six sunflower genotypes, of which two were wild and four cultivated accessions, that are a part of a vast sunflower collection located at the Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS) were investigated. The study conducted within this project was divided into two parts. The first was carried out in order to determine the best water stress treatment. In this experiment, one wild and two cultivated accessions were analysed. The selected treatment was used, later on, in the second, main experiment in which we tested all sunflower genotypes. Genotypes were chosen based on phenotype variation caused by genotype by environment interaction in variable environmental conditions, particularly abiotic stress conditions of water deficiency. Root system of the majority of tested genotypes was previously phenotyped in the Growscreen-Rhizo System within COST action FA1306 under optimal growth conditions. Conducted research within the framework of the EPPN2020, together with the previously performed experiment, was the first of its kind conducted in sunflower root phenotyping by use of an automated platform. For more details see the video testimonial: https://eppn2020.plant-phenotyping.eu/Selected_Projects

The platform GrowScreen-Rhizo was validated for phenotyping a new plant species, sunflower and a protocol was adapted to applied drought stress on sunflower plants. One scientist from Serbia was trained in root and shoot phenotyping techniques during this access project. Four FZJ members (scientists and technical assistants) were involved in the performance of the experiment and the training. The results will be published in a pre-reviewed journal.

• WEROOT: Characterization of Wheat genetic Resources with high throughput root phenotyping to increase wheat adaptation to drought

The purpose of the project was the characterization of root traits of selected genotypes belonging to a Single Seed Descent durum wheat landraces. The plants were grown under two different conditions; for the drought conditions, the soil was pre-dried to 35% soil water content (SWC) and for control soil was kept at 75% SWC. 9 selected genotypes have been used with 4 replicates for each genotype and treatment. The plants were grown for approximately 4 weeks and root traits were analysed every second day. The software used for images analysis allowed to extracte visible root traits, such as root length and spatial distribution of roots. In addition, shoot traits, such as plant height, number of leaves and tillers were combined with measurements of biomass and stomatal conductance. The root system was washed and scanned using WinRhizo scanners to quantify total root length. This procedure allowed to assess whether the image acquisition provided a reliable representation of the root systems under control and drought conditions. A high positive correlation ($R^2=0.89$; P< 0.001) was recorded between visible roots and total roots, validating this method as an effective root biomass predictive tool. Overall the drought treatment reduced shoots and roots, while the above ground part was more sensitive than the below ground part which is evident in the ratio of root to shoot which increased under drought condition. The experiment has enabled to identify genotypes that presumably could be tolerant or sensitive to drought.

One scientist from Italy was trained in root and shoot phenotyping techniques during this access project. Four FZJ members (scientists and technical assistants) were involved in the performance of the experiment and the training. A manuscript presenting the results will be submitted to the pre-reviewed journal Frontiers soon.





 HYPEROOTS: Testing root hypergravitropism as a trait affecting drought tolerance in barley

The aim of the project was to analyze the ability of three hypergravitropic barley (Hordeum vulgare L.) mutant lines to better cope with drought stress when compared to wild type (Morex cv.). The aim of this experiment was to test if barley lines showing enhanced root gravitropic growth provide any advantage in terms of tolerance to reduced soil water availability. The three mutant lines (egt1a, egt1 b, and egt2) have been identified within the chemically (NaN3) mutagenized barley population TILLMore, in the Morex cv. genetic background (Talamè et al. 2008; Bovina et al. 2011). In the GrowScreen-Rhizo platform, plants were grown for 3 weeks under three different conditions:

- control level: rhizotrons filled with 'well-watered soil' (70% water content);

- drought condition: rhizotrons filled with 'dry soil' (54% water content);

- semi-drought condition: 'dry soil' (54% water content) at the top of the rhizotron and 'wellwatered soil' (70% water content) at the bottom. The semi-drought two layer setting was meant to mimic common field drought conditions with available deep-soil water.

Roots and shoot traits were collected every second day. At the end of the experiment, destructive measurements were conducted to analyze root and shoot dry weight and shoot relative water content. Root images were analyzed following protocols available at GrowScreen-Rhizo platform (Nagel et al. 2012). All quantitative measurements and comparisons were evaluated following a statistical approach. On the basis of the results obtained, the mutants show considerable differences regarding total visible length of the roots, as well as for root angle (see Figure). Strategies of the mutants to cope with the drought conditions are not yet clear.

In this access project, FZJ tested successfully for the first time a two layer approach with layers with different water content within one rhizotron ('semi-drought' condition). As this condition mimics field situation, the established protocol will be very valuable for future phenotyping studying the effect of drought stress using rhizotrons. Two scientists from Italy were involved in this access project and trained in root and shoot phenotyping techniques. Four FZJ colleagues (scientists and technical assistants) were involved in the performance of the experiment and the training. Part of the results has been published in PNAS: Kirschner *et al.* 2021 (10.1073/pnas.2101526118). A second manuscript will be resubmitted to PNAS soon.



Figure: Root phenotype of egt2-1. Wild-type and egt2-1 roots grown in soil-filled rhizotrons of the installation GrowScreen-Rhizo differed especially in the root angle (Kirschner *et al.* 2021).

• RootPhenAlgo: Development of a fully automated root image segmentation software Characterizing the root system is a complex task due to its hidden nature. Some methods already exist to enable the visualization of the root system like the rhizotrons available in GrowScreen-Rhizo 1 platform (Nagel et al 2012).





Today there is no software which enables an automatic analysis of roots growing in soil environment. There are tools which allow a semiautomatic analysis such as Smartroot (Lober et al 2011) or Rootnav (Pound et al 2013). However, none of these is working without user inputs or manual annotations / corrections. Therefore, the challenge is to develop algorithms which permit to increase the throughput in root image analysis, with the objective in the best case to reach fully automatic root detection and extraction of root traits. Towards this aim, the main bottleneck of a full automation is to achieve a perfect segmentation of the root system from the background.

Agro Innovation International is the innovation and research division of the Roullier Group. It has a long expertise in the study and the development of efficient fertilizers improving crop growth. The R&D Centre (Centre Mondial de l'Innovation Roullier, Saint-Malo, France), which opened 3 years ago includes state of the art equipment in various fields: molecular biology, biochemistry, chromatography, mass spectrometry, microscopy and agronomy with 1200 m² of greenhouse facilities including a phenotyping platform. Agro Innovation International develops for its own purposes and for sharing with academic partners new algorithms for image analysis. However, although the new algorithms based on artificial intelligence are extremely powerful, they require a lot of annotated data to be developed. This proposal aimed to design a fully automated root segmentation and analysis software based on our algorithms and data and expertise of Jülich Plant Phenotyping Center. This TA project has lead us to new perspectives in plant science research, especially for root monitoring. Indeed, for a R&D Centre specialized in plant nutrition, the characterization of the root system is a must have to better understand the plant behaviour under different stimuli and under new fertilizers.

The rhizotron images of the platform GrowScreen-Rhizo had been used within this project to test new algorithms for root image analysis. One scientist from the industry partner Agro Innovation International has been introduced in the challenges of root images. Two FZJ scientists were involved in the TNA project. As the time of the TNA project was too short to develop a new algorithm for root image analysis, no publication will follow directly, but ideas developed during this project will be integrated in future publications.

• RHIZOTOX: Characterising autotoxicity tolerance in crops releasing natural herbicides.

To suppress competitors, some plant species have evolved a biochemical strategy called allelopathy: these 'donors' secrete herbicidal chemicals ('allelochemicals') to inhibit neighbours. Allelopathy can be exploited in agriculture to combat weeds because many crops have the capacity to synthesize such allelochemicals. However, a major caveat of allelopathic crops is that allelochemicals can also inhibit the growth of the donor, a phenomenon known as 'autotoxicity', with consequences for crop growth and yield. While little is known about the mechanisms by which donors counteract autotoxicity, understanding these processes is crucial to efficiently use these endogenous weed suppression strategies in sustainable agricultural practices.

The overall aim of the project was to identify the genetic factors that confer tolerance to autotoxicity in barley (*Hordeum vulgare*). Barley is an ideal model to screen for allelochemical autotoxicity tolerance: it produces many effective allelochemicals. The alkaloid gramine is synthesised by *Hordeum* species and varies in abundance among barley cultivars. In two pilot experiments using soil-filled rhizotrons and pots the application of gramine was tested. However, as it was not possible to develop a standardized protocol for gramine application within the framework of the TNA project, the gramine treatment was replaced by root phenotyping of a selected barley panel under control conditions and the data were combined with previous shoot data.

The platform GrowScreen-Rhizo was tested for the first time to study autotoxicity tolerance in crops. However, the planned treatment had to be skipped because of challenges in the solubility of used alkaloid. Due to Covid-19 pandemic the partners from Austria could not join the experiments in Jülich, but the experiments were performed in close collaboration. Four FZJ





members (scientists and technical assistants) were involved in the performance of the experiment. The results will be published in a pre-reviewed journal.

SAPHE: High throughput phenotyping of durum wheat accessions under salt stress Abiotic stresses, and especially salinity, represent a grave threat to agriculture dramatically affecting the crop production around the world. Drought and salinity are actually widespread in many regions around the world and are expected to increase rapidly reaching the salinization of more than 50% of the arable lands by the 2050. To develop new lines suitable for stress conditions it is important to understand the mechanisms involved in the salt tolerance, but also to know if there are differences among varieties since different varieties have different root architecture. The aim of this study was to evaluate salt effects on the root system among six contrasting durum wheat genotypes. In order to choose a stress level sufficiently high to cause a visible effect within a few weeks of treatment, but not too strong to kill the plants, two pilot experiments were performed, each approx. 3.5 weeks. As a result, it was decided to run the main experiment using the two treatments of 0 and 500 mM NaCl. The experiment was run with 72 rhizotrons using the GrowScreen-Rhizo 1 platform in a Randomized Block Design with 6 replications, 6 genotypes and 2 salinity levels for around 4 weeks. Plant material consisted of six durum wheat accessions: J. Kethifa (highly salt tolerant), Cham1 (moderately salt tolerant), and four lines that are also under evaluation in the framework of the ECOBREED Horizon 2020 project with contrasting root angles, Pelsodur, Vulci, Azeghar, and Sebatel. Main experiment results showed that the salt stress effect on durum wheat root-traits was significant, varied between genotypes, and over time. These results can help to select tolerant and susceptible genotypes at the seedling stage and identify promising durum wheat parental lines to insert in future breeding programs to create lines able to tolerate salt stresses.

The platform GrowScreen-Rhizo was validated for the first time to study the effect of salt stress in crops and new protocols for the application of salt stress using rhizotrons were successfully established. Due to Corona pandemic, the partners from Italy could not join the experiments in Jülich, but the experiments were performed in close collaboration. Four FZJ members (scientists and technical assistants) were involved in the performance of the experiment. The results will lead into a manuscript which will be published in a pre-reviewed journal.

3.1.2. PlantMRI

• PoTuWa : Isohydry of potato plants and tuber water capacitance

The Project aimed to better understand water relations of potato plants growing in drying soil. While the potato tuber is a large (starch) storage organ, currently very little is known about the role of the tuber as a possible water source under drought stress. Therefore the project investigated above- and below-ground plant and soil water relations using novel MRI technology paired with physiological measurements of the shoot. The project started in February 2019. Seed tubers were sent from Lancaster to Jülich (to ensure continuity with previous work at Lancaster) and a preliminary experiment planted on 26th April 2019. The first visit of the researcher from Lancaster was in June 2019 for 10 days to run a pilot experiment, optimise the workflow and prepare the two main experiments. Some pitfalls in the experimental planning were identified and solutions for the main experiments were discussed. Two experiments were conducted in summer 2019 and 2020. Each experiment imposed two treatments at 6 weeks after emergence: well-watered controls and plants that were allowed to dry the soil. During the growth period, weekly MRI images were taken to describe the growth pattern of the tubers. From the MRI images, tuber volume and tuber water content could be extracted using the software package MeVisLab. Since MRI is a non-destructive method, it was possible to measure the same plants every 4 hours (including night hours) over a time course of 4 days. Shoot physiological measurements were carried out twice daily (30 minutes before the plant underwent MRI imaging). Soil water content was measured at three heights of the pot twice daily, as well as whole plant and leaf transpiration rate, leaf photosynthesis





rate and stomatal conductance. All leaf level measurements were conducted on the same leaf on both measuring occasions each day, with its water potential and area determined in the afternoon of that day. Soil water content of both treatments was similar during the first 4 hours of the experiments, but re-watering of well-watered plants at 6 hours resulted in significant differences as soil water content declined in the drying treatment significant differences between treatments one day after imposing stress of drought stressed plants is already significantly smaller than in well watered plants in the first night after imposing drought stress. This unique dataset indicates that soil drying restricts tuber growth even before shoot physiological measurements detected differences, and well before visible leaf wilting occurred (not detected in this study). This finding can inform irrigation scheduling on farms in the future to prevent yield losses through drought stress.

The platform plantMRI was validated for the first time to study the effect of drought stress on potato tuber volume dynamics and the approach was successfully established. Three FZJ members (scientists and technical assistants) were involved in the performance of the experiment and a PhD student as well as supervisor from University of Lancaster were involved. Currently we are writing up the manuscript which will be published in a peer-reviewed journal.

• RooDy: Root Dynamics Under Varying Water Supply

Hydropatterning is a phenomenon consisting in the preferential growth of lateral roots in wet areas of the soil [1]. The objective of this experiment was to induce this phenomenon by creating conditions of soil moisture heterogeneity in a soil profile divided in four layers by vaseline barriers. This was possible thanks to rhizons inserted in each layer, which were used to inject water pulses. We also wanted to test whether this phenomenon becomes reversible whenever the soil moisture conditions change, namely if lateral growth is interrupted in the previously moist layer and promoted in a newly wetted layer. Zea mays was the species used in the experiment, which consisted of two treatments and one control, each one of them having seven replicates. The plants were kept in a growth room at 23°C and were exposed to a light/dark period of 10 and 14 hours, respectively. Layer 1 and layer 2 were the layers subjected to water injections, in the treatments. The pulses consisted in rewatering only the layer of interest up to 15% for four consecutive days. One layer received the first water pulse and the other layer received the second water pulse after the volumetric water content (VWC) halfed in the previously wetted layer. The controls were kept at 15% VWC in each layer for the entire duration of the experiment. The Soil Water Profiler (SWaP) was used every day to measure soil moisture in each layer and to determine how it varied due to root water uptake. The columns were weighed every day to calculate transpiration. Shoot length and leaf number were also measured. The MRI was used to image the root systems non-destructively every night in order to observe the root development over time and if the roots were responding to the treatments. At the end of the experiment leaf area was measured and the roots were collected destructively from each soil layer and scanned for a final measurement in length. The data collected have not been fully analysed yet. However, it was proved that the vaseline barriers are a good method for creating an hydraulic isolation between soil layers. We also observed preferential lateral root growth in wet soil areas and we noticed that the differences in water uptake rates between layers appeared to be consistent with the differences in root abundance between layers. At first look, it appears that the root system started to grow more laterals in the soil layer wetted by the first water pulse as soon as two days after its application, as visible in the left-hand side of Fig. 1. This behaviour continued until when the VWC in that layer halfed and the second pulse was applied in the other layer. At this point, there seems to be an interruption in root growth in the firstly wetted layer and a promotion in root growth in the newly wetter layer, as visible in the right-hand side of Fig. 1.







Fig. 1: Succession of MRI images taken every forty-eight hours. An increase in lateral growth was observed in layer 2, forty eight hours after the start of the pulse. 1. Fort-eight hours later starting applying pulse 2 it appears to be an interruption in growth in layer 2 and promotion in root growth in layer 1.

The experimental approach will be described and published in a technical paper in a biological open access journal, e.g. frontiers in plant science or AoB Plants. Depending on the clarity of the results, they will be published as an article in a biological open access journal as mentioned above, or a more general scientific journal reaching a wider audience. Along with the open-access publication of the papers, the corresponding data and code will be published on open access archives (e.g. zenodo.org), whereas the entire computational workflow including scripts used for the generation of plots will be publicly shared on platforms such as renkulab.io and zenodo.org. This will maximise verifiability and re-usability of the results, and promote open science in experimental biology.

References: [1] Fromm, H., 2019. Root Plasticity in the Pursuit of Water. Plants 8, 236.

Overall this project looked at the effect of reduced water availability in the soil on root growth. In order to do this a 45cm soil column was divided into four hydraulically separated parts where water could be added by means of rhizos. The plantMRI platform was used to observe root growth over a four week period, 15 plants being scanned every second day. Water pulses were administered based on soil water content (SWC) measurements performed with the SWaP (van Dusschoten, 2020) to be able to quantify the reduction SWC per compartment. Root growth can be determined based on differences in root lengths on separate days and changes of the root growth can be observed in relation to the duration since the water pulse was supplied and simultaneously the SWC was observed. This should allow to determine the correlation between root growth dynamics and SWC dynamics. At the time of writing of this report no final conclusions can be drawn as the experiment was finished at the end of September 2021 and data analysis is in full progress. However, first course analysis does indicate that root growth varies with locally available SWC. The main issue with this experiment involved timing as the used maize plants would need to withdraw water from the individual compartments in a way that allows one to observe effects in a matter of three to four days. Therefore pre-experiments were required to estimate the required plant size, which determines transpiration rates. Based on MRI images that were generated on the fly, root development in the compartments could be observed whereby the starting point of water pulses could be determined. In combination with the SWaP measurements timing of pulses could be optimized.

Three scientists and a technical assistant from FZJ were involved, a Ph.D student, his supervisor and three technical assistants from LIST were involved. Training was received by





the Ph.D student from LIST. At this stage it is unknown whether or not the data allows for a publication, even though initial analysis looks promising.

 Root-to-Pore: How does root systems adapt to pore networks in compacted soils? – Impacts on root system architecture and energy cost of barley

This project aimed to understand 1) how the root system architecture and 2) the energy cost of barley crop are responding to complex soil environment in compacted conditions. The project was funded by the EPPN2020 Transnational Access to Research Infrastructures, and the research activity was carried out in Forschungszentrum Jülich, Germany for four weeks (from the 15th of September to 15th of October, 2021) according to the schedule (Fig. 1).



Fig. 1 Schedule of planned experiments and analysis in Root-to-pore

This part of the work used the non-destructive magnetic resonance imaging (MRI) method in the Enabling Technologies group in Institute of Bio- and Geo-Sciences, IBG2, to track the development and growth of barley (*Hordeum vulgare* cv. Barke) roots in compacted and non-compacted soils with and without soil pore networks. Briefly, we artificially manipulated soil pore network in soils (Fig. 2), and examined to what extent the plant root system would preferably explore these non-resistance areas in soil (as opposed to the compacted soil with maximum physical resistance), and whether this will influence the water and nutrient uptake.



Fig.2 Experimental design of Root-to-pore approach with (a) control- and pore treatment (b,c)

Confirming the hypothesis, root developed faster and deeper in compacted soils with pore network (Fig. 3a) and, on average, more than 50% of the seminal roots were in the soil pores. On the contrary, root development and growth were strongly limited in compacted soil without soil pores (Fig. 3b) In this case, roots were shorter, thicker when comparing at the same days after sowing (DAS), and the tortuosity of root system was higher, indicating the physical resistance during penetrating soil volume.







DAS 3 DAS 7 DAS 9 DAS 12 DAS 3 DAS 7 DAS 9 DAS 12 Fig. 3 The development and growth of barley root (*Hordeum vulgare* cv Barke) in compacted soil (bulk density: 1.6 g/cm³) with soil pore network (a) and without pores (b) at 3, 7, 9 and 12 days after sowing (DAS)

This project will be followed-up to evaluate the energy spending during root growth using isothermal calorimetry in Sweden, and we plan to publish the results in an international journal. During the preparation of the manuscript, we will acknowledge the funding from EPPN2020 by stating that "Financial support by the Access to Research Infrastructures activity in the Horizon2020 Programme of the EU (EPPN2020 Grant Agreement 731013) is gratefully acknowledged".

Overall, in this innovate approach plantMRI was used to study the effect of pores and soil compaction on growth and root system architecture of plant roots. Novel technological approaches for establishing stable pores and specific densities for soil compaction was applied. Initial results indicate that the pores allow for an increased growth rate of roots to forage into depth, especially in high density soils. Less anticipated was the reduction of lateral root development in the upper part of the root system. Initial analysis showed no change of the shoot biomass and leaf area and the presence of pores, even if they induced a considerable change of root system architecture.

Three FZJ members (scientists and technical assistants) were involved in the performance of the experiment and a postdoc of the guest university were involved. Currently complementary measurements quantifying energy consortium of roots growing in pores and different compacted soils are performed. This will be done with funding outside of EPPN2020. Afterwards we are writing up the manuscript which will be published in a peer-reviewed journal.

3.1.3. BreedFACE

 FUTURE-LEGUME: Effects of FUTURE atmospheric elevated CO2 on LEGUME nutrition, growth and molecular profiling

Nowadays humanity is facing one its biggest challenges: climate change. One of the main offenders is increased CO2 emissions. In 2016, atmospheric CO2 reached levels of 400 µmol mol-1 and is predicted to rise to 550 µmol mol-1 by 2050. It is now irrefutable that elevated CO2 (e CO2) will impact the nutrition of the foods which we will consume in the future, particularly legume crops which provide a large share of the global population diet, also being a crucial source of protein and minerals for human nutrition. Regarding this EPPN 2020 program the purpose of the performed work was to study the genetic variability of two crop species grown at ambient CO2 (aCO2, 400 µmol mol-1) or eCO2 (600 µmol mol-1) under BreedFACE experimental facility in Campus Klein Altendorf. Searching of genotypes which





have greater yield at eCO2 could support in adaptation to the future CO2 environment levels. Although intraspecific difference in responses to eCO2 has been found in several species, intraspecific differences in crop yield responses to eCO2 under field conditions have occasionally been documented. In this study, the responses of leaf photosynthesis, growth and yield parameters to eCO2 were examined in several genotypes of common bean and soybean under field conditions. Consequently, eCO2 significantly stimulated leaf photosynthetic net CO2 assimilation rate, but decreased light induced fluorescence transients in both species. Moreover, CO2 enrichment significantly increased plant leaf area, aboveground dry biomass and seed yield in both species. Bean seed yield at eCO2 range from 0.75 to 2.12 times (mean 1.34) that at aCO2 in different varieties, and soybean seed yield at eCO2 range from 1.07 to 1.86 times (mean 1.51) that at aCO2 in the different varieties. The change in the number of pods at eCO2 was the primary determinant of the response of seed yield ranging from 0.88 to 1.89 times (mean 1.37), and 1.03 to 2.56 times (mean 1.71) in common bean and soybean genotypes, respectively. Our results indicate that significant variation in the response of seed yield to eCO2 under field conditions does exist among varieties of common bean and soybean, and that variation in the response of pod and seed number may be more important than variation in photosynthetic response.

seed number may be more important than variation in photosynthetic response.

The results thus far were used for two publications:

Quirós-Vargas, J., R. D. Caldeira, N. Z. d. Santos, L. Zimmermann, B. Siegmann, T. Kraska, M. W. Vasconcelos, U. Rascher and O. Muller (2021). Response of Bean (Phaseolus vulgaris L.) to Elevated CO2 in Yield, Biomass and Chlorophyll Fluorescence. 2021 IEEE International Geoscience and Remote Sensing Symposium IGARSS.

Soares J., L. Zimmerman, N. Zendonadi dos Santos, O.Muller, M.Pintado, M.W. Vasconcelos (2021) Genotypic variation in the response of soybean to elevated CO2. Plant-Environment Interactions *in press*

Further publications may follow from this dataset. In the experiment the visiting scientist (J.Soares) was trained in plant (physiological) field experiments. Additional student (N. Zendonadi, Juan Quiros, L.Zimmermann) were trained in soybean performance under elevated CO2. We were successful in growing common bean and soybean in the region the breedface was located though climate conditions may not by fully optimal for soybean and potential constrain a future experiment.

• HYSTORYCO2: The influence of elevated atmospheric [CO2] on physiology and yield of wheat cultivars accounting for 60 years of northern-European breeding

The increase in atmospheric carbon dioxide concentration [CO2] is one of the most evident climatic changes of the past ninety years (Trend in Atmospheric Carbon Dioxide 2019). It has been well established that the steady rise in [CO2] will significantly influence crop production worldwide. The primary effects on crops include stimulation of photosynthesis by the 'fertilization influence' particularly in C3 crops such as wheat (Ainsworth and Long 2005). At the same time, elevated [CO2] reduces stomatal conductance (gs) and transpiration (due to reduced stomatal aperture and a reduction in stomatal density). It has been proposed that in many species the increases in leaf temperature due to changes in gs may be detrimental to photosynthesis and could alter whole-plant water use efficiency (Osborne 2016; Gray et al. 2016). Wheat, a key food crop globally, is predicted to be greatly influenced by the rise in [CO2] and several reports suggest positive benefits in both yield and physiological performance (Christy et al. 2018). Several studies have demonstrated a strong correlation between gsand grain yield in crops (Roche 2015, Faralli et al. 2019a) grown at present atmospheric [CO2], including wheat Fischer et al. 1998). These studies have illustrated that both stomatal control of CO2 uptake for photosynthesis, evaporative leaf cooling and canopy temperature can account for these correlations. At the same time, evidence suggests that the compromise of reducing transpiration to save water might impact on plant performance through increasing leaf temperature Fischer et al. 1998). Therefore, it has been speculated that partial stomatal closure triggered by elevated [CO2] might negatively impact wheat production under specific





environmental conditions (Osborne 2016; Gray et al. 2016). The detrimental effect of a potential increase in leaf temperature following elevated [CO2] has not been extensively explored, although recent work suggests that even leaf temperatures of 25°C can inhibit photosynthesis in plants acclimated to cooler temperatures (Yamasaki et al. 2002) such as wheat. Breeding for yield improvement in wheat and subsequent assessment of physiological traits have been predominantly conducted at current ambient [CO2]. There is evidence to suggest a significant increase in gshas been the inadvertent result of breeding efforts in the last 70 years, however, this has never been demonstrated specifically in European and/or northern-European cultivars (see Roche 2015). Therefore, the aim of this study was to re-evaluate these key physiological traits under elevated [CO2] using FACE technology. Winter bread wheat cultivars representing 60 years of breeding were grown under current ambient and elevated [CO2]. We hypothesized that the physiological response of photosynthesis and gs to increasing [CO2] in north European wheat was dependent on the year of release.

In this experiment 3 researcher from Essex visited the Breedface facility. Furthermore the historic collection planted was used for student course at the university of Bonn in which plant traits were quantified from several proximal-remote sensing sensors. First year results were marginal in response to elevated CO2 which may have been related to the very hot summer therefore a second year to repeat the experiment was requested. Field experiments are typically constrained when only 1 year of data are involved to test for consistency of the results.

• PRIFACE : Influence of elevated CO2 concentration on potato plants, late blight resistance, and effectiveness of plant resistance inducers (PRIs)

Climate change will bring challenges to agriculture, and one concern is that plant protection measures to safeguard food security will be affected. In the guest to reduce fungicide application [1], the so-called plant resistance inducers (PRIs) can be employed to boost the plant's own immune system. But their performance in a future climate with elevated CO2 (eCO2) remains unanswered [2]. Recent studies on plant-pathogen interactions under eCO2 show that responses to CO2 vary according to the pathosystem. In this project, we suggested the study of potato late blight (PLB), caused by the oomycete Phytophthora infestans, which is by far the most serious potato disease worldwide. The spraying of fungicides and the introgression of resistance genes from wild Solanum species are the most common strategies of disease control. P. infestans has a great capacity of developing resistance to fungicides, as already shown formetalaxyl [3], and can break down plant resistance in a few growing seasons [4]. Integrated disease management is therefore highly encouraged, with PRIs as a good alternative since they work indirectly by controlling the pathogen through the plant's own immune system, thus having less impact on the environment. The effects of eCO2 on the efficiency of PRIs in controlling plant disease remains untested and was the main goal of this project. We proposed the use of Free-Air CO2 Enrichment (FACE) at BreedFACE, Forschungszentrum Jülich, to assess the efficiency of β-aminobutyric acid (BABA) and potassium phosphite on two potato cultivars, Bintie and Désirée, susceptible to PLB, as well as to study P. infestans ability to infect potato plants under eCO2. Visual scores were used for calculating the area under the disease progress curve (AUDPC), which will be used for determining the efficiency of the PRIs by checking their relative control of PLB compared to untreated plots. We also took additional measurements of the canopy using UAV-based spectral imaging and fluorescence measurement techniques (FloX, Fluorescence Box, and LIFT, Light-Induced Fluorescence Transient) to estimate the photosynthetic efficiency of the plants and estimate different vegetation indices related to plant biophysical properties such as chlorophyll and water content. Leaf samples were also sampled for further molecular analysis to identify how differences in gene expression and protein/metabolite composition between PRI-treated and untreated potato plants alter resistance to P. infestans in both atmospheric CO2 (aCO2) and eCO2. This project provides valuable data towards a more holistic research approach by incorporating different types of data (e.g., sensor-based and -omics) that may indicate specific signalling compounds or pathways to be targeted, which





could improve not only the selection of crops with higher inducibility and better resilience to climate change but also the development of better disease management strategies.

In this experiment one researcher from SLU was intensively trained in plant field phenotyping under elevated CO2 while he brought the knowledge of potato late blight to students in Juelich. The relatively wet year 2021 resulted in a good spread of potato late blight that during dry years may have been difficult. The potatoes were harvested one week before the due date of this report and hence results still need to analysed to prepare for publications.

• HYSTORYCO2_II: The Influence of Elevated [CO2] on physiology and yield of wheat cultivars accounting for 60 years of northern-European breeding.

Atmospheric CO2 concentration ([CO2]) is predicted to rise significantly in the next decades leading to changes in wheat (Triticum aestivum L.) physiology and productivity. Evidence suggests that important physiological traits linked to wheat yield such as stomatal conductance (gs) and photosynthetic capacity will be influenced by elevated [CO2]. Although it has been shown that wheat yield will benefit from the elevated [CO2] fertilization, the influence on stomatal behaviour, transpiration and leaf temperature needs further characterization (Osborne 2016; Gray et al. 2016) Breeding for genetic gain and assessment of physiological traits for wheat improvement have been predominantly carried out at ambient [CO2]. Therefore, the aim of the proposed work is evaluate key physiological traits and yield components of winter bread wheat accounting for 60 years of breeding subjected to ambient and elevated [CO2]. A significant increase in gs was shown due to breeding efforts in the last fifty years for Central American spring bread wheat that resulted in increased transpiration and water use. However, to date this trend has not been established in European and/or northern-European cultivars with the exception of some work carried out in a panel of Italian durum wheat. Therefore, we hypothesize that different sensitivity to the increasing [CO2] in north European wheat is dependent on the year of release. In addition, due to the emerging potential source limitation for both grain number and grain weight determination recently highlighted, a significant diversity for leaf and spike photosynthetic stimulation, yield and yield components increase under elevated [CO2] is hypothesized, which will also be assessed. The proposed physiological assessment will cover a range of traits including leaf photosynthesis and transpiration, leaf-to-canopy temperature, stomatal density, biomass accumulation and other remote-sensing techniques.

The work will set the basis for exploring potential novel physiological targets for inclusion in future wheat breeding programmes focusing on the yield optimization under the predicted future elevated [CO2]. In addition, the experiment will provide a comprehensive physiological basis of bread wheat in relation to the breeding history and elevated [CO2] conditions thus linking yield components, physiology and potential occurring interactive trade-offs.

The second year of growing the historical wheat collection coincided with the Corona pandemic such that the researcher from Essex could not visit this year. The winter wheat was grown and harvested by Juelich and a selection of detailed measurements were done next to the inclusion into remote sensing monitoring. The breedface was again subject to research question of students of the master course of University Bonn, who among others studied the effect of plant height (taller old varieties versus shorter new varieties) in relation to Light induced fluorescence transient measurements. Publications in prep.

3.1.4. GrowScreenChamber

• Phenotypic Variation: Studying the Epigenetic Landscape of Different Populations of Arabidopsis thaliana through their phenotypic variations

This phenotyping experiment was designed to investigate the effect of Epigenetic Variation (EV) on phenotypic diversity using several genotypes (accessions) of Arabidopsis thaliana. In order to study such effects, on the one hand, we generated populations with different levels of standing EV using distinct propagation histories. And on the other hand, all individuals of single





population were initially grown from the same plant to ensure genetic homogeneity. This method of propagation allows us to interpret the observed phenotypic variation in a constant environment as a result of different levels of standing EV; and with further investigations we aim at confirm this at the molecular level.

We expect lower levels of EV in populations where individuals were continually derived from a single parent (Single Seed Descent, SSD) than in populations where individuals were derived from many parents (after the initial single-parent stage; Population Seed Descent, PSD). Additionally, for the Epi-Hybrid Population (EHP), individuals were obtained from crosses between genetically identical PSD individuals; they are thus expected to have the highest level of EV due to the possible additive behavior of parental EV. Moreover, the EHP will also allow us to investigate potential heterosis effects that have a purely epigenetic basis.

This access project was carried out by a PhD student, Ms. Hoda Mazaheri, University of Zürich, supervised by Prof. Ueli Grossniklaus. Continuous practical and technical assistance throughout the experiment was provided by FZJ-IBG-2 personnel, namely three platform technicians and one automation engineer, and by two scientists, Dr. Fabio Fiorani and Dr. Nathalie Wuyts. Provided training included: a) use of the automation portal of the platform; b) RGB image segmentation and quality control; c) data preparation for statistical analysis. The experiments did not require specific technical adaptations to the platform and there were no resulting publications so far.

OASES: Characterisation of splicome function in Arabidopsis tolerance to water stress Drought is one of the main environmental adverse conditions limiting crop yield worldwide. Understanding the molecular mechanisms controlling plant response to water limitation is essential if we want to obtain new crop varieties with increased tolerance to this adverse situation. It is well known that plant response to water stress is tightly controlled by changes in gene expression. Recently, the modulation of splicing has emerged as a new layer of regulation in the control of stress-induced gene expression. We designed this project to study the role of different core components of the spliceosome, the macromolecule that catalyze splicing reaction, in the regulation of plant adaptation to water limitation. We have characterized the tolerance to drought of 23 Arabidopsis mutants with altered expression of these intermediaries. For this characterization, we have monitored different traits (i.e. plant size, weight, photosystems status by chlorophyll flourescence, etc.) of wild-type and mutant plants growth under control conditions or under severe irrigation limitation. The analysis of the data obtained will give us very interesting data no only to understand the molecular mechanisms controlling plant adaptation to abiotic stress, but also to increase our knowledge about the regulation of the splicing process.

This access project was carried out by a senior scientists, Dr. Rafael Catala, University of Madrid. Continuous practical and technical assistance throughout the experiment was provided by FZJ-IBG-2 personnel, namely three platform technicians and one automation engineer, and by two scientists, Dr. Fabio Fiorani and Dr. Nathalie Wuyts. Provided training included: a) use of the automation portal of the platform; b) RGB image segmentation and quality control; c) data preparation for statistical analysis. The experiments did not require specific technical adaptations to the platform and there were no resulting publications so far.

• PLC-Drought: The role of PLC in drought tolerance

We are studying the role of phospholipase C (PLC) in plant stress signalling and development. Overexpression (OE) of PLC has been shown to enhance the drought tolerance of maize, rice, tobacco and canola (Wang et al. ,2008; Georges et al.,2009; Tripathy et al., 2012), and our lab has recently found this for three independent Arabidopsis PLCs (Zhang et al., 2018a,b, van Wijk 2018). To increase our understanding of the molecular mechanism by which PLC improves drought tolerance, we have overexpressed three additional PLC genes (i.e. 6 out of 9 AtPLCs total), and created multiple transgenic lines that express AtPLC5 driven by 13 different cell/tissue-specific promoters. The Phenotype platform is used to characterize them.





This access project was carried out by a PhD student, Mr. Max Van Hooren, University of Amsterdam, supervised by Prof. Teun Munnik. Continuous practical and technical assistance throughout the experiment was provided by FZJ-IBG-2 personnel, namely three platform technicians and one automation engineer, and by two scientists, Dr. Fabio Fiorani and Dr. Nathalie Wuyts. Provided training included: a) use of the automation portal of the platform; b) RGB image segmentation and quality control; c) data preparation for statistical analysis. The experiments did not require specific technical adaptations to the platform and there were no resulting publications so far.

• PhenoArHeat: Phenotyping of BPM1-mediated heat stress response in Arabidopsis thaliana

The main purpose of the proposed experiment was to characterize transgenic Arabidopsis lines with modified protein ubiquitination and DNA methylation processes, and their response to heat stress. To achieve this goal, a total number of 1840 plants was characterized. The number reported was the result of the number of genotypes proposed (23), the number of groups tested for each genotype (4), and the number of replicates for each genotype and group tested (20). Moreover, at least two growth chambers were necessary because of simultaneous profiling of a control (24 °C) and heat treatment group (40 °C). Based on the number of plants and the experimental design, we needed and used two growth chambers within the GrowscreenChamber platform, equipped with cameras for RGB and fluorescence imaging.

This access project was carried out by a PhD student, Ms. Sandra Vitko, University of Zagreb, supervised by Prof. Zeljka Vidakovic-Cifrek. Continuous practical and technical assistance throughout the experiment was provided by FZJ-IBG-2 personnel, namely three platform technicians and one automation engineer, and by two scientists, Dr. Fabio Fiorani and Dr. Nathalie Wuyts. Provided training included: a) use of the automation portal of the platform; b) RGB image segmentation and quality control; c) data preparation for statistical analysis. The experiments did not require specific technical adaptations to the platform and there were no resulting publications so far.

4. Reflection on results of the TNA programme

4.1.1. GrowScreenRhizo

In general the users of the six TNA projects using the GrowScreen-Rhizo installation addressed quite diverse research questions. This diversity allowed to test quite different approaches and methods. The TNA projects enabled to validate the GrowScreen-Rhizo platform for new crop species, to establish and modify standardized protocols for different stresses, and to adapt the system to simulate field conditions. In addition, new approaches for improving root image analysis in future have been evolved. New collaborations have been established within Europe which resulted so far in one common project application between users and TNA provider. Furthermore, the outcome will be joint publications. One paper has been published already in a high ranging journal (PNAS) and another five publications are in preparation or planned. This outcome could be achieved by intense discussions with the users starting already during the application process as well as by selection of scientific relevant projects by external reviewers. Some of the rejected proposals of one call were revised and re-submitted in a follow-up call which further improved the quality of the TNA projects.

4.1.2. PlantMRI

In two of the three projects the experiments didn't initially run as planned. Even with some prior pilot experiments unexpected surprises occurred, like the size of the plants being thus that the number of plants to be investigated had to be reduced. Also, climatisation of the plants turned out to be quite critical as plants needed to be in the same room as the plantMRI in order to prevent climatic shocks for the plants. As a climated chamber has become available ext to the





MRI and we were able to use it in a limited fashion, the quality of the measurements, which are dependent on the climatic stability the plants experience, increased significantly. The use of the climate chamber in combination with the MRI will be very important for future projects. Also, in order to increase the potential success rate of projects, well performed pilot experiments are required and should be an integral part of project planning, as these short visit do not allow for failed experimental runs.

4.1.3. BreedFACE

The BreedFACE allowed growth of three different crops under elevated CO₂ in the field wihtin EPPN2020. In Germany winterwheat was grown previously whereas beans and potatoes were grown, to our best of knowledge for the first time in a Free Air CO2 Enrichment (FACE) conditions. Between crops and within different crop varieties the response to elevated CO_2 differed varying from marginal to no increase of yield in winterwheat and significant increase in biomass in beans. Further plant traits were quantified non-invasively using the Fieldsnake, a custom made vehicle that can position sensors above the plots within the area where CO_2 is elevated. Together these installations allowed field phenotyping under elevated CO₂ successfully throughout the four years of EPPN2020 access. The different researchers involved in the BreedFACE through EPPN2020 increased the knowledge of specific crops and their a(biotic) response. Besides the specific research questions, as described in the project summaries, also students of four master courses benefited from the unique experiments and used data of the additional sensors (e.g.from UAVs) and or performed small experiments within. For future access programmes multi-year access modes can be considered as the environmental variation in the field would need confirmation of at least one year repeat, ideally more.

4.1.4. GrowScreenChamber

We completed four access project, as initially planned. All four projects resulted in relatively large experiments using the capacity of the installation to about 80%. From a technical standpoint we had to face a major technical problem during one experiment, which required the repetition of this experiment. Otherwise, no specific adaptations or developments were required to perform the experiments. In all four projects hundreds of plant tissue samples harvested at specific time points depending on the various treatments and experimental protocol were shipped to the users for subsequent molecular analyses. Reflecting on the experience of the provided access, the demand for the use of phenotyping platforms fully dedicated to the model plant Arabidopsis remains relatively high. However, it was somewhat surprising that the offered capacity was not utilized by flagship Arabidopsis genetics projects in Europe. Reaching specific groups of European Arabidopsis geneticists could represent a target for future experiments involving the use of the GrowScreenChamber installation.

5. References

Avramova V., Nagel K.A., AbdElgawad H., Bustos D., DuPlessis M., Fiorani F., Beemster G.T.S. (2016) Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *Journal of Experimental Botany*, 67(8), 2453-2466, doi:10.1093/jxb/erw055.

Bovina R, Talame V, Ferri M, Tuberosa R, Chmielewska B, Szarejko I, Sanguineti MC (2011) Identification of root morphology mutants in barley. *Plant Genet Res* 9:357-60.

Gioia T., Nagel K.A., Beleggia R., Fragasso M., Ficco D.B.M., Pieruschka R., De Vita P., Fiorani F., Papa R. (2015) The impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilisation. *Journal of Experimental Botany*, 66, 5519-5530, doi:10.1093/jxb/erv289.





Kirschner G.K., Rosignoli S., Guo L., Vardanega I., Imani J., Altmüller J., Milner S.G., Balzano R., Nagel K.A., Pflugfelder D., Forestan C., Bovina R., Koller R., Stöcker T.G., Mascher M., Simmonds J., Uauy C., Schoof H., Tuberosa R., Salvi S., Hochholdinger F. (2021) ENHANCED GRAVITROPISM 2 encodes a STERILE ALPHA MOTIF-containing protein that controls root growth angle in barley and wheat. *Proceedings of the National Academy of Sciences (PNAS)*, 118 (35) e2101526118; DOI: 10.1073/pnas.2101526118

Lobet, G., Pagès, L., & Draye, X. (2011). A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiology*, 157(1), 29-39.

Nagel K.A., Putz A., Gilmer F., Heinz K., Fischbach A., Pfeifer J., Faget M., Bloßfeld S., Ernst M., Dimaki C., Kastenholz B., Kleinert A.-K., Galinski A., Scharr H., Fiorani F., Schurr U. (2012) GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. *Functional Plant Biology*, 39, 891-904.

Pound, M. P., French, A. P., Atkinson, J. A., Wells, D. M., Bennett, M. J., & Pridmore, T. (2013). RootNav: navigating images of complex root EPPN 2020 RootPhenAlgo - ID: 329 6 of 6 architectures. *Plant Physiology*, 162(4), 1802-1814.

Soares J., L. Zimmerman, N. Zendonadi dos Santos, O.Muller, M.Pintado, M.W. Vasconcelos (2021) Genotypic variation in the response of soybean to elevated CO2. Plant-Environment Interactions *in press*

Talamè V., Bovina R., Sanguineti M.C., Tuberosa R., Lundqvist U., Salvi S. (2008) TILLMore, a resource for the discovery of chemically induced mutants in barley. *Plant Biotechnol J* 6:477–485.



