



COST Action FA1306:
The quest for tolerant varieties –
Phenotyping at plant and cellular level



COST WG1 / EPPN2020 workshop **29th - 30th of September 2017**

Novi Sad

Abstract book

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Scientific Programme

Friday, September 29th

8:00 – 9:00	Registration
9:00 – 9:05	Welcome
9:05 – 9:20	IFVCNS movie
9:20 – 9:45	Roland Pieruschka: <i>Integrating plant phenotyping community in Europe: EPPN2020: access to phenotyping facilities EMPHASIS: long term operation of pan European phenotyping infrastructure</i>
9:45 – 10:30	Key lecture: Hendrik Poorter: <i>Pampered inside, pestered outside? Ways to bridge the gap between lab and field experiments</i>
10:30 – 10:45	Coffee Break
Session 1: Phenotyping/Breeding for biomass improvement Chair: Roland Pieruschka	
10:45 – 11:00	Koller: <i>Non-invasive phenotyping technologies enable investigating plant responses to antibiotic and biotic interactions</i>
11:00 – 11:15	van Rooijen et al.: <i>High throughout phenotyping of photosynthesis and growth to identify relevant genetic loci in Arabidopsis</i>
11:15 – 11:30	Onno Muller: <i>BreedFACE: phenotyping for plants under elevated CO₂ concentrations</i>
11:30 – 11:45	Sciara et al.: <i>High-throughput phenotyping of vegetative growth and water-use efficiency of durum wheat near isogenic lines for QYLD.IDW-3B, a major QTL for yield per se</i>
11:45 – 11:55	Ljubičić et al.: <i>Normalized Difference Vegetation Index (NDVI) as a tool for wheat yield traits estimation</i>
11:55 – 12:05	Hernandez et al.: <i>Quantization of Harvested Tomatoes from RGBD Images</i>
12:05 – 12:15	Herrera et al.: <i>How can drones and modern phenotyping methods contribute to the understanding of Genotype × Environment interactions (G × E)?</i>
12:15 – 12:25	Szabados et al.: <i>A non-destructive method to monitor plant growth and development in vitro</i>
12:25 – 12:35	Sekmen et al.: <i>The Role of Hydrogen Peroxide in Elongation Dynamics of the First Internode of a Wheat Cultivar Tolerant to Deep-Sowing Condition</i>
12:35 – 13:30	Lunch
13:30 – 14:30	Poster session
Session 2: Phenotyping/Breeding for nutrient efficiency Chair: Astrid Junker	
14:30 – 14:45	Junker et al.: <i>Integrated analysis of plant growth and development using high throughput multi-sensor platforms at IPK</i>

14:45 – 15:00	Salon et al.: <i>High throughput root phenotyping using the “Rhizo” suite</i>
15:00 – 15:15	Pastor et al.: <i>Relationship between liposoluble fingerprints and botanical origin of various agricultural crops</i>
15:15 – 15:30	Lillemo et al.: <i>Reliable and efficient high-throughput phenotyping to accelerate genetic gains in Norwegian plant breeding</i>
15:30 – 15:45	Vasconcelos et al.: <i>Phenotyping soybean and common bean for better growth and nutrition under elevated CO²</i>
15:45 – 16:15	Coffee Break
Session 3: Phenotyping/Breeding of perennial crops Chair: Rick Van de Zedde	
16:15– 16:30	Gonçalves & Martins: <i>An efficient phenotyping for selection in ancient grapevine varieties</i>
16:30– 16:45	Svensgaard et al.: <i>Phenotyping of perennial ryegrass by physiological fingerprinting and UAV remote sensing using RGB-, thermo- and multi-reflectance imaging</i>
16:45– 17:00	Corke et al.: <i>Breeding perennial species to enhance the sustainability of grassland based agriculture</i>
17:00– 17:15	He et al.: <i>Novel 3D Imaging System for Strawberry Phenotyping</i>
17:15– 17:30	Paudel et al.: <i>Heavy soil and treated waste water result in reduced hydraulics and reduced levels of plasma membrane aquaporin (PIP) mRNA in citrus trees</i>
17:30– 17:45	Costa et al.: <i>Dynamics of canopy and soil temperature variation in a Mediterranean vineyard</i>
20:00 – 23:00	Gala Dinner

Saturday, September 30th

8:30 – 9:00	Papa: <i>The impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilisation</i>
Session 4: Phenotyping/Breeding for biotic stress tolerance Chair: Diego Rubiales	
9:00 – 9:15	Boureau et al.: <i>Quantification of biotic stresses on aerial parts of plants using Chlorophyll Fluorescence Imaging and Image Analysis</i>
9:15 – 9:30	Rubiales: <i>Resistance to rusts: can we predict durability by complementation of field and growth chamber studies with histology?</i>
9:30 – 9:45	Costa et al.: <i>Phenotyping Castanea hybrids from controlled crosses for resistance to Phytophthora cinnamomi</i>
9:45 – 10:00	Aznar-Fernández & Rubiales: <i>Phenotyping Pisum sativum germplasm for resistance to aphid (Acyrtosiphon pisum) and weevil (Bruchus pisorum) under field and controlled conditions</i>
10:00 – 10:15	Svara et al.: <i>Polyploidy influences Malus x domestica / Venturia inaequalis interactions</i>

10:15 – 11:00	Coffee Break
Session 5: Phenotyping/Breeding for abiotic stress tolerance Chair: Sebastien Carpentier	
11:00 – 11:30	Pauk et al.: <i>Phenotyping for drought tolerance in wheat using complex stress diagnostic system</i>
11:30 – 11:45	Welcker_ et al.: <i>A genome-wide approach combining field and platform phenotyping to investigate plant responses to drought and high temperature</i>
11:45 – 12:00	Rakosy-Tican et al.: <i>Phenotyping preselected somatic hybrids of potato with the wild species <i>Solanum chacoense</i> and <i>S. bulbocastanum</i> for drought tolerance</i>
12:00 – 12:15	Carpentier et al.: <i>High throughput growtainer phenotyping combined with on line transpiration monitoring to select water efficient cultivars: a proof of principle in the banana bio(di)versity collection.</i>
12:15 – 12:30	Paul et al.: <i>Synergistic effects of salt and drought stress in wheat responses studied by high throughput phenotyping</i>
12:30 – 12:45	Sundgren et al.: <i>Root growth and anatomy of spring wheat in response to waterlogging</i>
12:45 – 13:00	Nazemi et al.: <i>Molecular mapping of root traits in durum wheat in environments with lower water availability</i>
13:00 – 13:15	Aliferis: <i>Functional genomics and phenomics: focusing on plant protection products' R&D</i>
13:15 – 13:30	Baytar et al.: <i>Association mapping for fiber traits and drought tolerance in elite cotton (<i>Gossypium hirsutum</i> L.) germplasm</i>
13:30 – 13:45	Ottosen et al.: <i>Phenotyping for heat tolerance - from lab to field</i>
13:45 – 14:00	Moshelion & Dalal: <i>Whole-plant stress performance analysis: a new tool for functional phenotyping</i>
14:00 – 14:30	Lunch
15:00 – 18:00	Excursion

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Preface:

Integrating plant phenotyping community in Europe and beyond

Roland Pieruschka, Ulrich Schurr

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Quantitative analysis of structure and function of plants has become a major bottleneck in research and applied use of plants. Approaches targeting relevant traits are needed to quantitatively address key processes and understand the dynamic interactions between genetic constitution, molecular and biochemical processes with physiological responses leading to the development of phenotypes. To address this bottleneck a close interaction within the community is required.

The talk will illustrate the role of plant phenotyping networks, summarize the recent activities and provide an outlook for future ones. Within Europe the COST action (FA1306: The quest for tolerant varieties - Phenotyping at plant and cellular level) has established a important discussion between scientists with expertise on divers topics related to plant phenotyping. The recently funded EU project EPPN2020 enables practical access to plant phenotyping facilities in Europe and fosters essential steps to develop and implement phenotyping pipelines. The ESFRI listed project EMPHASIS aims at long-term sustainable development of the plant phenotyping infrastructure in Europe. Finally, the International Plant Phenotyping Network, a non-profit association integrates the plant phenotyping community as a global communication hub.

Key lecture:

Pampered inside, pestered outside? Ways to bridge the gap between lab and field experiments

Hendrik Poorter

Plant Sciences (IBG2), Forschungszentrum Jülich, Germany

In this talk I discuss how representative plants grown under controlled conditions (growth chamber, glasshouse) are for those growing in the field. A meta-analysis showed that lab-grown plants had faster growth rates, higher N concentrations, and higher SLA. They remained smaller, however, because lab plants are grown for much shorter time. We compared glasshouse and growth chamber conditions with those in the field and found that the ratio between the daily amount of light and daily temperature (photothermal ratio) was consistently lower under controlled conditions, especially for experiments with *Arabidopsis*. This may strongly affect a plant's source: sink ratio and hence their overall morphology and physiology.

Plants in the field also grow at higher plant densities. A subsequent meta-analysis showed that a doubling in density leads on average to 34% smaller plants with strong negative effects on tiller or side-shoot formation and yield per plant, moderate effects on allocation and photosynthesis, but little effect on plant height or reproductive effort. We found the r^2 between lab and field phenotypic data rather modest (0.26), although still larger than the across-genotype r^2 for year-to-year variation in yield in the field (0.08). Based on these insights, I discuss various alternatives to facilitate the translation from lab results to the field, including options to apply growth regimes closer to field conditions.

- Poorter et al. (2016) Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol.* 212: 838.
- Poorter et al. (2012a) The art of growing plants for experimental purposes: a practical guide for the plant biologist. *Funct. Plant Biol.* 39: 821.
- Poorter et al. (2012b) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol.* 39: 839.

Session 1: Phenotyping/Breeding for biomass improvement

Oral presentations:

Non-invasive phenotyping technologies enable investigating plant responses to antibiotic and biotic interactions

Robert Koller

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Individual plants vary in their capability to respond to environmental changes. The plastic response of a plant determines the ability to mitigate environmental constraints, ensuring growth and reproduction and thus their agricultural success.

Plant phenotyping aims at providing quantitative and novel traits of plants, which are critical in responding to dynamic changes of the environment and may help to improve our knowledge on plant growth and yield. This needs measurements of large numbers of plants and plant parameters, because phenotypic responses are continuous and mostly non-linear in space and time.

For a holistic characterization, IBG-2 develops and provides non-invasive 2D and 3D imaging technologies for quantitative analyses of plant above and below-ground structure and function. This characterization will help to select traits and genotypes sensitive for abiotic and biotic interactions in agrosystems.

Our facilities include field installations to investigate the response of crop species to elevated CO₂ (BreedFACE), growth chambers with controlled environmental conditions designed for routine phenotypic evaluation of shoot parameters of rosette plants (GrowscreenChamber), rhizotron installation which allows simultaneous and non-invasive phenotyping of root and shoot growth and architecture (Growscreen-Rhizo) and a Magnetic Resonance Imaging (MRI) installation to measure dynamic changes of roots in a 3D soil environment (PlantMRI).

We will present an MRI case study that illustrates various aspects non-invasive root phenotyping and its potential application for breeding towards biomass improvement. Overall, non-invasive phenotyping technologies enable investigating plant responses to abiotic and biotic interactions and we are convinced that this approach will accelerate crop improvement in a modern crop management.

High throughput phenotyping of photosynthesis and growth to identify relevant genetic loci in Arabidopsis

Roxanne van Rooijen^{1,2,4}, Pádraic J. Flood^{1,2,5}, Aina Prinzenberg^{1,2}, René Boesten¹, Lucía Campos Dominguez¹, Willem Kruijer³, Fred van Eeuwijk³, Jeremy Harbinson², and Mark G.M. Aarts¹

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Natural genetic variation in plant photosynthesis efficiency is hardly studied even though breeding for photosynthesis would be interesting to maintain increases in crop yields. One of the reasons this is not studied is the notorious difficulty in adequately phenotyping photosynthesis parameters for genetic research. We designed a versatile plant phenotyper, the Phenovator, capable of multispectral imaging of 1440 plants per experiment. Imaging includes chlorophyll fluorescence to determine the light use efficiency of photosystem II electron transport (Φ_{PSII} or F_q'/F_m'), and near infrared reflection to measure projected leaf area. This system has been used to phenotype *Arabidopsis thaliana* recombinant inbred line populations as well as around 350 genetically diverse accessions for genome wide association analysis. Plants were phenotyped at optimal conditions and in response to cold treatment (5 °C) or a switch from 100 to 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance. The observed genotypic variation was used to identify quantitative trait loci (QTL). Our latest progress in identification and confirmation of QTL for Φ_{PSII} , plant growth and epinastic leaf movement will be presented, including detailed analysis of the allelic variation at the nucleotide level of some of the genes underlying these QTL. Our work has shown that there is sufficient genetic variation for PSII efficiency in *Arabidopsis* amenable for gene identification, which suggests the same will be the case for crop species. Such would offer interesting opportunities for future crop photosynthesis, and subsequently yield, improvements.

BreedFACE: phenotyping for plants under elevated CO₂ concentrations

Onno Muller¹, Angelina Steier¹, Laura Freiwald¹, Einhard Kleist¹, Beat Keller¹, Lars Zimmermann^{1,2}, Thorsten Kraska², Uli Schurr¹ and Uwe Rascher¹

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Worldwide CO₂ concentrations will continue to increase and alter regional weather patterns substantially accordingly (IPPC 2014). Elevated atmospheric CO₂ concentrations can stimulate photosynthesis and induce stomatal closure affecting carbon uptake and water use. However little is known about the interaction between CO₂ and abiotic/biotic constraints and about the genetic variability of the response to its atmospheric concentration. Thus, the development of strategies for adaptation and acclimation of crop productions to changing environmental conditions requires large-scale studies in all fields of plant research and production.

Systems to study elevated CO₂ on plants grown under open field conditions, so called Free Air CO₂ Enrichment (FACE) systems, have proven their relevance in providing information in conditions closer to agronomic situations than plants grown in greenhouses and open top chambers. The German plant phenotyping network (DPPN) therefore established the BreedFACE for breeding new crop varieties with higher yield and better stress performance at future CO₂-concentrations.

BreedFACE is a unique combination of mobile CO₂ storage containers to allow potential placement in breeder's and agronomic relevant fields. In 2017 BreedFACE is set up and tested in the field lab Campus Klein-Altendorf of the University of Bonn. A population of winter wheat grown in the field, which was later, in part, substituted, by soy and common bean. The test phase of the BreedFACE did not allow for continuous supply of CO₂ to the crops but valuable information is obtained for future operation. Here we will report on the operation of the BreedFACE and its integration with phenotyping and standard agronomic practices.

The BreedFACE is a key component of the field phenotyping module set up in DPPN and access to it is provided through the European Plant Phenotyping Network (EPPN). This infrastructure for growing crops at elevated CO₂ will be combined with state-of-the-art field phenotyping equipment using fixed, mobile and flying platforms to observe plants's performance.

High-throughput phenotyping of vegetative growth and water-use efficiency of durum wheat near isogenic lines for QYLD.IDW-3B, a major QTL for yield per se

Giuseppe Sciara ¹, Silvio Salvi ², Claude Welcker ², Llorenç Cabrera Bosquet ², Marco Maccaferri ¹, Antonin Grau ², François Tardieu ², Roberto Tuberosa ¹

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In this study, we used the phenotyping platform PhenoArch in order to identify growth and water use related traits that may explain the segregation for yield and plant height observed at *Qyld.idw-3B*, a major QTL for yield per se (Graziani et al. 2010). Four pairs of durum wheat near-isogenic lines (NILs) for *Qyld.idw-3B* were grown at three levels of drought stress: no stress (soil water potential > -1 bar), mild stress (soil water potential of -5/-8 bar) and severe stress (soil water potential of -13 bar). The stress was applied at the beginning of stem elongation until the end of the experiment (late milk stage, Zadok 77) on eight replicates per genotype per treatment. We recorded two main types of phenotypic data: (i) canopy images and (ii) weight measurements: every night, digital RGB images were collected. From these images we estimated several growth related phenotypes like biomass, leaf expansion and plant height; every plant was weighted to estimate the evapotranspiration at least once per day. Combining these data, it was possible to evaluate key physiological parameters like water use efficiency (WUE) and leaf transpiration. The QTL did not affect vegetative growth and water use of plants during the early vegetative stages while showing strong differences between NIL pairs during the reproductive and early ripening stages. This explains the segregation for final plant height previously observed by Graziani et al. (2010). These results may provide useful information for further phenotypic as well as physiological and genetic characterization of the effects of *Qyld.idw-3B* with a main focus on mid to late stem elongation and reproductive/maturity stages.

Normalized Difference Vegetation Index (NDVI) as a tool for wheat yield traits estimation

Nataša Ljubičić¹, Radivoje Jevtić², Sanja Brdar¹, Oskar Marko¹, Marko Panić¹, Marko Kostić³,
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Field-based high-throughput plant phenotyping using spectral reflectance measurements possess the great potential to improve genetic gains for different yield traits of wheat. The objective of this study was to estimate the potential of using NDVI (Normalized Difference Vegetation Index) measurements of wheat crop canopy in identification of a specific growth stage in which remotely sensed data shows the highest correlation with aboveground biomass, grain weight per spike and final grain yield of 28 winter wheat genotypes. The NDVI was determined using an integrated proximal sensor Green-Seeker (NTech Industries Inc., Ukiah, California, USA) and hyperspectral camera (Ximea Corp., Lakewood, CO USA) at five development stages of wheat: full flowering (BBCH 65), medium milk (BBCH 75), late milk (BBCH 77), early dough (BBCH 83) and fully ripe stage of wheat (BBCH 89). Overall 26 hyperspectral NDVI were calculated from two-band combinations between red (600-700 nm) or far red (700-740 nm) and near-infrared (756-946 nm) regions. The relationships between examined traits and NDVI readings at different development stages were determined using Pearson correlation coefficient. Obtained results indicated that NDVI values depend upon the particular phenology stage. Wheat genotypes differed in the decline of NDVI from full flowering to fully ripe stage of wheat. While high yielding genotypes maintained high NDVI values, lower yielding genotypes expressed steep descent. Highly significant correlations (higher than 0.7 and significant at $p < 0.05$) were found between the specific hyperspectral NDVI indices at medium milk stage and all examined yield traits of wheat. The strong positive relationship between NDVI and examined traits found at medium milk stage of wheat implies that this stage is optimal for wheat traits assessment in semiarid conditions or similar wheat growing environments. Results also indicate that significant differences between NDVI values obtained through Green-Seeker sensor and hyperspectral camera at the most comparable spectral band combination were not observed. This suggests that both can be used for the assessment of aboveground biomass, grain weight per spike and final grain yield of a large number of wheat genotypes in rapid and non-destructive manner. Additionally, our study reveals that richer information from hyperspectral camera provides alternative spectral combinations that can be utilized for more precise phenotyping.

Quantization of Harvested Tomatoes from RGBD Images

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Plant phenotyping is a required task in order to accomplish the best possible results in plant breeding. New proposed industrial technological methods such as image processing and image analysis are bringing promising improvements to plant phenotyping. However, these methods mainly have been used under laboratory conditions. There is a strong demand to apply them in natural environments where the plants either are too large to be transported or have to remain in their natural habitat. In this study we present a use case of industrial technologies to improve plant phenotyping where these technologies have to be implemented in agricultural environments, such as greenhouses, to provide evidence-based results on measuring plant phenotypic traits. The image processing and analysis methods were applied directly in a greenhouse by bringing a RGBD camera mounted on robotic platform to tomato plants. Tomato fruits were measured on two consecutive days before and after harvesting. The difference between these measurements is compared against the total weight and count of the harvested fruits.

A high-resolution industrial camera (Raytrix, 7.25 megapixels) with Light Field technology was used in the image acquisition system. The camera included a micro lens array, which transformed it from a normal camera into a RGBD (Red, Green, Blue and Depth) single lens camera. The Light Field raw data can be processed to obtain the 2D RGB images and the 3D depth maps allowing the adjustment of the focus and point of view of a recorded image. The image acquisition system was placed on the Phenobot Phenotyping Robot at Enza Zaden Company in Enkhuizen, Netherlands.

A set of 1343 RGBD images were obtained from 52 plants, uniformly distributed among 17 plots before harvesting. A second set with the same number of images was obtained for all plots right after harvesting. Harvesting data included the number of fruits per plant and total weight of fruits per row section. Tomato cluster matching and image mosaicking were used to create a single image per plot from all the input images. The Harris corner point's detection algorithm was used in combination with RANSAC method to determine the projective matrices to match the tomato clusters. The watershed algorithm and the detection of the sepals were used to segment individual tomatoes from the fruit clusters.

From qualitative analyses, the implemented algorithms for tomato segmentation, sepal segmentation, fruit counting, cluster matching and image mosaicking performed properly. However, the correlation calculation was affected by the low quality of a number of the depth images.

How can drones and modern phenotyping methods contribute to the understanding of Genotype × Environment interactions (G × E)?

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Genotype performance varies across environments, leading to variance differences and rank changes among genotypes. As a result, many countries conduct genotype trials to characterize the performance of genotypes as a function of sites. A better understanding of genotype by environment interactions is fundamental for predicting accurately genotypic performance in farms and increasing our knowledge to develop future crop varieties.

In 2016, Agroscope, the Swiss federal center for agricultural research, started to evaluate the use of unmanned aerial vehicles (UAVs) and specific ground phenotyping devices in its official variety trials with the objective to better characterize genotypes and to obtain parameters for the prediction of genotypes' performance at a national scale. The objective of this contribution is to share first results and experiences of this initiative using results of winter wheat as an example.

We evaluated ground sensors as tools to increase the accuracy and assessment's speed of variables such as plant density and number of tillers per area. In addition, we tested a fix wing UAV and a multicopter equipped with RGB, multispectral and thermal sensors. Although both types of UAVs had advantages and disadvantages, we found that the multicopter was more suitable for the objectives of our research. The different sensors, on the other hand, allowed estimating spectral indexes that characterize traits associated to phenology, senescence, and tolerance to water limitations and heat stress. We transformed the information given by the spectral indexes into parameters that relate the value of the trait with grain yield for critical growth phases of wheat. We used these parameters together with limiting factors that account for the effects of environmental variables on wheat productivity to develop a predictive model of winter wheat genotypes in unobserved sites. Prediction accuracy, evaluated through correlations between predicted and observed grain yield ranged between $r=0.10$ and $r=0.80$. Although, our conclusions are based on preliminary results, these results show that indexes derived from UAV's and new phenotyping methods have potential to improve models that predict genotypic performance in unobserved environments.

A non-destructive method to monitor plant growth and development *in vitro*

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Non-destructive analysis of plants through color imaging is an increasingly popular method to define growth parameters, characterize plant development in time. High throughput automatic phenotyping systems can analyse environmental responses of multiple genotypes in a reproducible way. Cost of such platforms is however high, and is not affordable for many researchers. Low cost methods are usually destructive, more time consuming and can measure one particular feature of the plants.

We have developed a non-invasive method to simultaneously measure morphological and physiological parameters of *in vitro* cultured Arabidopsis plants. Plant growth is monitored by repeated color imaging with a commercial digital camera using neutral white background. High-resolution photographs are analysed by a MatLab-based computer application PlantSize, which simultaneously calculates several morphological and physiological parameters including rosette size, convex area, convex ratio, chlorophyll and anthocyanin contents. In the present configuration the software performs fast and simultaneous analysis of up to 36 Arabidopsis plants, exporting numerical data on an MS Excel-compatible format. Utility of the system is demonstrated by revealing small but significant differences between wild type and transgenic plants overexpressing the HSFA4A transcription factor or the *hsfa4a* knockout mutant, subjected to different stress conditions. While HSFA4A overexpression was associated with better growth, higher chlorophyll and lower anthocyanin content in saline conditions, the mutant showed hypersensitivity to stresses. Differences in development pattern were revealed by comparing rosette size, shape and color of wild type plants with phytochrome B (*phyB-9*) mutant.

The developed technology offers a simple, affordable and fast way to measure size, shape and color of Arabidopsis plants. The methods are based on non-destructive imaging allowing repeated measurements and monitoring changes of various growth parameters in time.

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The Role of Hydrogen Peroxide in Elongation Dynamics of the First Internode of a Wheat Cultivar Tolerant to Deep-Sowing Condition

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H₂O₂ is known as a multiple signaling molecule in plant cells, but its role in the elongation of the first internode, which is one of the tolerance strategies of plants to deep sowing conditions, is not yet clear. For this aim, in the present study, how H₂O₂ production, redox regulation and the activities of antioxidant enzymes are affected in their first internode of “Tir” seeds-imbibed with H₂O₂ under shallow- and deep-seeding conditions (2 cm and 10 cm, respectively) was investigated. It was found length of the first internode was increased in the H₂O₂-treated wheat seedlings at the depth of 10 cm. As seen by quantitative RT-PCR, H₂O₂ up-regulated cell wall loosening-related genes (*Glucanase EI* and *TaEXPB23*) in the first internode of “Tir” under deep sowing condition (10 cm). Additionally, H₂O₂ also up-regulated *RBOHD* and *RBOHF* genes (respiratory burst oxidase homolog genes) resulting in an increased in NOX (NADPH oxidase) activity. This is the first report that deep-seeding and H₂O₂ together induces the NADPH oxidase (NOX) activity and activities of antioxidant enzymes (SOD, POX, APX and GR). However, neither exogenous application of H₂O₂ nor deep-seeding did cause any damage in the cell membrane of the first internode, as evident by decreased lipid peroxidation. As a result, this study shows that, under deep-seeding condition, low concentration of H₂O₂ induces the activities of antioxidant enzymes to trigger of antioxidative response in the first internode elongation of “Tir”. Thus, H₂O₂-mediated first internode elongation under deep-seeding condition is correlated with regulating the concentration of H₂O₂ through increased activities of antioxidant enzyme and expansin/glucanase-mediated cell-wall loosening.

Poster presentations:

Integrating omic approaches for sustainable genetics resources' uses in Brassica breeding programmes

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The material importance of the characterisation and utilisation of genomics and genetics resources for food and agriculture can be conceived in terms of the value in achieving three transitions: (a) Genomics to plant biology; (b) Genomics to improved crops and (c) Genomics to society. The role of the biosciences in the use of genetic resources understands how plants function and it can be used to support improved plant breeding.

Metabolomics is the large-scale study of metabolites produced by a species. Metabolic analysis can be considered as a range of technical approaches such as the determination of a group of components within a pathway, the metabolite fingerprinting (direct comparison of plant samples) to metabolomics (qualitative/quantitative analysis of all metabolites present).

Metabolomic techniques are predicted to have major potential in providing molecular definition of complex quality attributes as the phytonutrient compounds glucosinolates (GLS). Portuguese National gene bank (BPGV) detains a *Brassica* collection of 1,261 landraces accessions (acc.). *B. oleracea* is the largest (741 acc.) followed by *B. rapa* (380 acc.) and *B. napus* (139 acc.). The *B. oleracea* collection contains 149 of acc. of the subspecies *costata* ("penca" and "tranchuda" landraces) and 573 are the kale crop type from the subspecies *acephala* ("galega" landraces with diversified designation).

The objective was the assessment of Brassica accessions, on the content in glucosinolates by liquid chromatography: twelve acc. (nine of *B. oleracea* and 3 acc. of *B. rapa*) were morphologically phenotyped and evaluated to glucosinolates (GLS). The sampling used for GLS analyses were 3 leaves per acc for *B. oleracea* species and for *B. rapa* a bulk from the 3 plants per acc of turnip greens was used.

The analysis of glucosinolates was carried out according to the method previously described for the assessment of intact glucosinolates. The quantified glucosinolates were tentatively identified according to the UV-spectra and data available in the bibliography on retention time and elution order and expressed in µg/g dry weight.

The glucosinolates identified were in agreement with those reported in literature to different Brassica species and subspecies. The GLS results showed the same results pattern as the phenotyping taxonomic results. The quantitative results showed genetic diversity for different Portuguese landraces. The galega landraces have more genetic diversity for the qualitative analysis; the profile of metabolites was different, for the different designations from this landrace. Combining field and laboratory knowledge can be useful for breeding Brassica crops strategies of food production and nutrient content.

Effects of light-emitting diodes and fluorescent light on growth and development of *Arabidopsis* plants

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Until today fluorescent, metal-halide and incandescent lamps have been used as supplementary lighting for crop production. Even though these sources induce an increase of daily photosynthetic flux levels, they are not as energetically efficient as desired. The use of light-emitting diodes (LEDs) brings several key advantages over existing illumination technologies for indoor plant cultivation including long life, small volume, low heat emission, adjustable light intensity, high energy-conversion efficiency and wavelength specificity.

For this study we selected the most important model plant, *Arabidopsis thaliana*, which is widely used in plant biology and genetics as well as in the study of plant-pathogen interactions. The research hypothesis is that cultivating of *Arabidopsis* seedlings under the varying irradiation wavelengths would differently affect morphological properties. The objective of this research was to investigate the effect of LEDs lighting on morphological characteristics of *Arabidopsis* cultivar “Columbia”.

The results of the experiment showed that the light regime had a significant effect on the following parameters: time of flower buds formation and flowering, length of main bolt and number of side bolts. In comparison to the control (fluorescent light), plants cultivated under LED light formed flower buds earlier and subsequently started flowering earlier. Additionally, they also have longer main bolts and higher numbers of side bolts.

On the other hand, LED light did not affect the time of seeds germination. There was also no difference between plants under fluorescent and LED lights regarding the length and width of plant leaves. The average number of leaves differed only at the late stage of vegetation and showed that LED plants formed slightly smaller number of leaves.

Based on our study we propose that LEDs are suitable for the indoor cultivation of *Arabidopsis* plants.

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Genotypic variation for NDVI and crop biomass at anthesis in six-rowed winter barley

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Crop biomass accumulated during pre-anthesis period is essential for maintaining high grain yield especially under conditions of various abiotic stresses. Fast and non-destructive estimation of crop biomass is important for cereal breeding since it could be utilized as an additional selection criterion. Among different spectral reflectance indices, Normalized Difference Vegetation Index (NDVI) represents one of the most widely used methods for estimation of crop biomass, yield and other physiological traits in different cereal crops. The main goals of this study were to analyse variation in NDVI values and crop biomass at anthesis and to estimate NDVI relationship with crop biomass in six-rowed barley genotypes. To this end, plant material consisted of different six-rowed winter barley genotypes was sown at an experimental field of the Institute of Field and Vegetable Crops, Novi Sad in randomized block design with three replications. Our result showed presence of significant genotypic variation in crop biomass and NDVI values at anthesis. Furthermore, crop biomass at anthesis was positively related ($r=0.42$) to NDVI values. These results indicate that NDVI measurements at anthesis could be used as a useful inexpensive screening method in breeding programs for rapid assessment of genotypes biomass.

Tropical pumpkin fruit phenotyping

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Cucurbita moschata Duch. Ex Poir. (tropical pumpkin) is widely distributed and cultivated in various agro-ecological conditions, especially in the tropical and subtropical areas. Tropical pumpkin has multiple uses in both food and pharmaceutical industry. In rural tropical areas it serves as household food security and is considered as the most important vegetable in traditional agricultural systems. The fruit is abundant in high stored carbohydrates and carotenoids, and contains moderate amount of vitamin C and some minerals. As, tropical pumpkin can be grown in different conditions, its genotypes significantly vary in yield and fruit quality.

Due to recent increased attention that tropical pumpkin has been receiving mainly because of the high nutritive and health value, we have performed morphological examination of the most important part of the plant - the fruit. Thirty-three fruit characteristics were used for evaluation of nineteen *C. moschata* genotypes that are a part of a big *Cucurbita* sp. collection of the Institute of Field and Vegetable Crops, Novi Sad. The analysed genotypes included the ones collected in Serbia, as well as genotypes of international origin. A great qualitative and quantitative variability was observed between the analysed genotypes, including fruit yield per plant that varied between 4.8 and 88.3 kg. The phenotypic evaluation gave an insight into the available variability and allowed identification of the desirable genotypes for future breeding, as well as selection of appropriate fruit traits to comply with the market demand.

Vintage phenotyping for modern breeding of false flax

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The industrial and technical-technological progress of modern society brings advantages and makes life much easier for people. However, with global climate change, this progress leaves less areas of fertile soil and reduces the planet's flora and fauna. This causes many problems, and one of the most important is preserving the existing sources of food and energy raw materials and finding new ones. Considering the incredible growth of the world's population, this is one of the greatest challenges of the modern population. The creation of more productive and nutritionally richer genotypes of agricultural plants would contribute to solving this problem. In order to obtain such genotypes, new approaches in identifying phenotypic markers were developed, in order to make it easier to scan the existing germplasm and selecting parental pairs. However, the use of advanced technology in phenotyping of agricultural plants is economically unjustified, especially for developing countries. Older and proven ways of phenotyping of cultivated plants provide good results to the breeders, the basis for the correct setting of the breeding target and the implementation of the program. This was confirmed by the results of the experiments on the species *Camelina sativa* L., set up in 2016 in the agroecological conditions of Vojvodina (Novi Sad, Rimski Šančevi).

False flax (*Camelina sativa* L.) is a plant of the family *Brassicaceae*, and its used one of the best natural sources of unsaturated fatty acids and also a biodiesel. Phenotypic variability of ten genotypes of false flax was examined. The evaluation of the variability, uniformity and stability of the genotypes was done according to the DUS test (Distinctness, Uniformity, Stability), which is in line with the technical procedures of the existing guidelines of the International Union for the Protection of New Varieties of Plants. Phenotyping involves five agronomic important traits of a false flax: the height of the plant, the height to the first side branch, the number of lateral branches, number of silique per branch and the number of seeds per plant. The results showed that the genotype CK1X-69 had the highest mean plant height (75.7cm) and the number of silique per branch (14.1), while the seed number per plant dominated the genotype CJ11X-43 (772.0). The height up to the first side branch had values of 32.77 cm (genotype CJ2X-9) to 46.40 cm (genotype CJ2X-80). The largest number of lateral branches was recorded for the CJ11X-52 genotype (7.73). The lowest variability in plant height was found in the genotype CJ11X-52, while in terms of the number of silique per branch and the number of seeds per plant, the lowest variant was the CK1X-25 genotype. For the other phenotypic markers, the lowest coefficient of variation was found in CJ2X-7 genotype. Depending on the observed phenotypic marker, Duncan's post-hoc test showed that the investigated genotypes were grouped into one or two groups, indicating their common geocenter of origin and uniform parental material from which they were derived.

The results of classical phenotyping of quantitative traits in combination with using molecular markers can help the breeders in selection of parental pairs and selection of desirable genetic variability.

The Finnish National Plant Phenotyping Infrastructure

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Current bio economy strongly relies on developing sustainable plant production for food, protein feed, wood fibre and biofuels. To promote these activities, fundamental and applied plant research should form a continuum allowing translating the vast amount of genomic knowledge to crop plant performance. To fill this gap, we have established a National Plant Phenotyping Infrastructure (NaPPI) at the University of Helsinki Viikki campus as a Finnish Research Infrastructure (FIRI) platform. The aim of NaPPI is to provide access for the plant research community to up-to-date phenotyping facilities. These high throughput facilities accommodate image based analysis tools for small herbs, berries and model plants such as *Arabidopsis*; for large crops, such as legumes, cereals, potatoes; as well as tree seedlings of spruce, pine, birch, and poplar. The system is based on automated plant management by weighing and watering, imaging by different sensors, and data acquisition provided by the Photon Systems Instruments (www.psi.cz). This system allows automated detection of growth and morphology, yield parameters, analysis of photosynthetic performance, as well as many physiological responses using RGB, infrared and fluorescence cameras. Here we report a research project on phenotypic screening of Turnip rape (*Brassica rapa* L. ssp. *oleifera*) breeding population for yield improvement. Turnip rape is an important oil seed crop in the northern hemisphere and as for many crops, nitrogen fertilization and hybrid breeding enhance stem elongation and thus risk of lodging. Semi-dwarf cultivars tend to lodge less allowing higher yield indices. Here, the dwarf gene BREIZH (*Bzh*) INRA was backcrossed into spring turnip rape, and the segregating F₂ population was analysed for growth and morphological parameters using imaging based phenotyping technology. Principal component analysis was used to identify automatically derived parameters that allow ranking the variation within the segregating population and cluster individuals in phenotypic groups. The obtained imaging based growth parameters were verified by manual measurements and finally the genotypes were confirmed by High Resolution Melting (HRM) analysis. Detection of the dwarf gene in homozygote state correlated with strongly reduced height caused by shortening of the internode length. The heterozygote population showed high variation in rosette area and the *Bzh* gene affected the number of branches and height, but didn't affect the number of nodes. These features together cause better lodging resistance of the semi-dwarfs.

Biomass dynamics and grain yield of triticale, barley and ryegrass in Mediterranean rainfed conditions

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Advances in plant breeding have made triticale and barley viable forage crops for dual-purpose uses (forage, hay and/or grain), specially during periods of animal food shortage in Portugal, under a strong Mediterranean influence.

This study was carried out to compare the agronomic traits: - dry matter accumulation, plant height, grain yield, hectoliter weight, number of grains per spike, 1000 kernel weight and forage quality traits: - crude protein content, neutral detergent fiber content (NDF) and in vitro digestibility, at three phenological stages (Zadoks growth scale: Z31: end of tillering; Z51: Beginning of heading; Z83: Early dough) of nineteen triticale genotypes, five barley genotypes and one *Lolium multiflorum* genotype during 2015-2016 at Elvas, which has a characteristic Mediterranean environment. In this study, it was also evaluated the capacity of re-growth between phenological stage Z31 and Z50.

Understanding the differences in dry matter and grain yield between these species could be useful for designing more sustainable extensive livestock system.

Analysis of variance showed highly significant differences ($P < 0.05$) among genotypes and for the majority of the agronomic and forage quality traits in the different phenological stages. Considering the first cutting (Z31), the average dry matter production was 1042 kg ha^{-1} , which represents an important source for livestock feed in winter. The highest protein content was found at this early stage of growth (21-27%). Significant genotype-to-genotype differences were found for NDF, with values varying between 37% and 65%. For Z53 and Z83 stages, the observed variation in NDF was higher than for the Z31 stage (from 45% to 64% and from 37% to 42%, respectively). For vitro digestibility, inter-genotypic differences were highly significant (73.7% to 78.8%) for each one of the cutting stage. The average grain yield of triticale was 4393 kg ha^{-1} . Barley reaches an average of 3121 kg ha^{-1} .

These results show the high potential of triticale and barley for extensive animal production and in the specific agro climatic conditions of south Portugal. These preliminary results indicate that our genotypes are suitable for double purposes uses (direct grazing, forage, hay and/or grain) to feed animals in extensive field conditions.

Effect of different treatments on the eggplant seed dormancy

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Eggplant is one of the plant species with initial seed dormancy directly after harvest. As a natural phenomenon, dormancy can be conditioned physiologically (embryo dormancy, endosperm and seed coat dormancy) and morphologically (morpho-physiological, physical and combined, or complex dormancy). This trial included 5 genotypes from germplasm collection of the Institute for Vegetable Crops in Smederevska Palanka in Serbia (L2, L7, L12, L33, and L34). Seeds were extracted from fruits on day 73 after flowering for all genotypes in the trial. Seed was then dried to 10% moisture. After 5 days the cooling treatments commenced. Cooling pre-treatment (HLS) was performed at +4°C for the following periods: 96 hours, 72 hours, and 48 hours. Next, the hormonal pre-treatment was performed using gibberellic acid (GA_3) in three different concentrations (5 ml/100ml, 15 ml/100ml, and 25 ml/100ml) for 24 hours. The effect of chemical pre-treatment with KNO_3 was also tested in three different concentrations (0.5%, 1%, and 1.5%) for 24 hours. Controls were not treated and their germination was monitored during storage period (after 3, 6, and 12 months). Seed germination was determined using the standard method. The results were processed using AMMI statistical model. Differences were found between genotypes and between treatments. The highest stability was found in genotypes L12 and L34. These two genotypes belong to weakly dormant seed. Cooling treatments showed different degrees of stability, and the highest stability coefficient (8.54) was found in the treatment with shortest cooling (48 hours). The treatment with 95 hours of cooling showed ASV coefficient of 5.15 which means it is a highly stable treatment.

Assessing genetic variability of Serbian and Austrian winter wheat varieties for pre-breeding

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It is generally agreed that further increase of wheat yield and stability can be ensured by broadening genetic diversity of elite wheat varieties. This can be achieved by enriching wheat gene pools used in a regional wheat breeding programme with germplasm from different regions that can be easily adapted to the local environment. Different wheat germplasm exploited in breeding programmes in continental Europe and Pannonian region reflects specific breeding requirements in distinctive environmental conditions. These factors contributed to development of allelic diversity and valuable genetic variation for most of the traits among wheat varieties grown in central and eastern European regions. In order to evaluate a potential of locally bred and grown wheat varieties in Austria, as a source of new genetic variability in a wheat breeding programme in Serbia, 42 elite winter wheat representatives of two different European breeding pools were assessed at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The genotypes were sown in a row-column design with three replications during 2016/2017 season and phenotyped for heading time, plant height, ear length, chlorophyll content and commonly occurring wheat diseases. Besides, the genotypes were analysed with microsatellite markers to estimate genetic diversity and determine population structure. A significant phenotypic variation in all traits was found. On average, the Serbian varieties had earlier heading date, shorter stems and ears, higher chlorophyll content and seemed to be more susceptible to leaf rust than the Austrian varieties. The Serbian and Austrian varieties were clearly differentiated by the principal coordinate analysis and the model-based clustering method with some sub-clusters within each group. The principal component analysis revealed general characteristics of the groups and also indicated varieties from different groups with similar heading date that could be used for crossing. In order to plan future breeding schemes for more adapted germplasm, a more detailed on-going research will complement these preliminary findings.

Variation in chlorophyll content in wheat near-isogenic lines for photoperiod response

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Chlorophyll content in leaves varies through different stages of plant growth and it is affected by their exposure to various abiotic and biotic factors. Changes in chlorophyll content can be easily measured by different non-destructive methods enabling monitoring of the plant state. For crops, this information can be used further to optimize grain yield in a specific region. Wheat is one of the most widely cultivated crops owing to its great genetic potential to synchronize anthesis with favorable environmental conditions. Photoperiod (*Ppd*) genes play an important role in accelerating or delaying flowering time in the spring after satisfying vernalisation requirement. Apart from that, *Ppd* genes have pleiotropic effects on some yield related traits. The material analysed in this study consisted of 10 modern Serbian wheat varieties and 57 NILs of variety Paragon differing in *Ppd-1* alleles - lines with introgressed single (16) and double (22) insensitive *Ppd-1* alleles, and 19 lines with single, double or triple knockouts of *Ppd-1* alleles. The genotypes were sown at the experimental field of the Institute of Field and Vegetable Crops, Rimski Šančevi, Serbia in 2016/17 growing season and plots were organized in the randomized block design with three replications. The chlorophyll content of the flag leaf was measured during two growth stages, at anthesis and grain filling period. Moreover, heading and flowering time were recorded. Analysis of variance revealed statistically significant differences among the groups of wheat genotypes for all analysed traits. The highest mean value for chlorophyll content was observed in the set of Serbian varieties, while the lowest was measured for the NILs with accumulated knockouts of *Ppd-1* alleles. Statistically significant negative correlations were observed between chlorophyll content and heading and flowering time in the all three sets of NILs. The former correlations were absent for the well adapted Serbian varieties that were significantly earlier regarding the heading and flowering time than the variety Paragon, from which NILs were created.

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Phenotypic characterization of the Serbian poppy (*Papaver somniferum* L.) population

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The poppy (*Papaver somniferum* L.) is used for oil and protein production from seeds, as food, in medicine (because it contains alkaloids of capsules - morphine, codeine, etc.), in cosmetics industry, in industry of colours, etc. The evaluation of poppy quality is generally based on the oil and morphine content. The experiment with poppy was conducted in four replications on the plots of the Institute of Field and Vegetable Crops, in Bački Petrovac (φN 45 ° 20 ', λE 19 ° 40', msl 89), in 2017. The aim of this experiment was to examine the morpho-productive properties of the Petrovac poppy population sown in spring. The examined poppy population had a round shape of the capsule. On average, plant height was 93 cm, the number of capsule in plant was 2.67, the capsule length was 35.70 mm, capsule width was 34.90 mm, and the capsule weight was 3.68 g. The seed weight in the capsule was 1.86 g while the 1000 seed weight was 0.34 g. The stability of the tested parameters measured by the coefficient of variation, recorded a low variation, which shows us that the examined population is a good base for stable production and for further work in the breeding of this oilseed.

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Relationships among oil content, protein content and grain yield in wheat (*Triticum aestivum*, L.)

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Assessment of health-beneficial chemicals in wheat grain and bran, such as proteins and oil is important for the breeding due to increased consumer demands for healthier food. Also, it is very important to know their relationships with the grain yield and to what extent they could be modified by growing conditions. The objective of this study was to investigate oil and protein contents, as well as grain yield of 25 winter wheat (*Triticum aestivum*, L.) varieties and to establish correlations among these traits in different environments. The field studies were performed on three different experimental sites in Serbia during two growing seasons (2009/10 and 2010/11). The investigated locations representing Serbia's most important wheat-growing areas: Rimski Šančevi (45°20'N, 19°51'E, 87 m altitude), Sremska Mitrovica (46°06'N, 19°33'E, 83 m altitude) and Pančevo (44°50'N, 20°40'E, 76 m altitude). The oil was extracted from wheat bran obtained by laboratory mill MLU 202. Classical Rushkovsky method was used to determine oil content, while protein content was determined by the ICC 105/2 method. Significant variability was found among the genotypes for all analysed traits. In the six environments, the oil content varied from 2.4% to 5.6%, with the average value of 3.8% and coefficient of variation (CV) 15.7%. The protein content ranged from 11.2% to 17.8% with the CV of 5.4%, while the grain yield varied from 2.3 to 9.8 t/ha, with the CV of 17.8%. The average genotypic values for all environments have shown negative correlation between protein content and grain yield ($r=-0.739^{**}$). This correlation was highly significant in three (E3, E4 and E6) out of six environments, while significant positive correlation between oil content and grain yield ($r=0.441^{*}$) was found only in E6. The established correlations between the traits are highly environmentally dependant and could be modified by variable growing conditions. It will be very valuable for breeding process to identify climatic variables and growing conditions that modify unwanted correlations, as well as to identify genotypes in which these correlations are not expressed in order to allow successful breeding for improvement of both traits at the same time. For positive correlations it is also useful to know the favourable conditions in which they can be maximized.

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project number TR31066) and by the COST Action FA 1306.

Phenotypic evaluation of variability among dry bean cultivars landraces from breeding collections of Institute of Field and Vegetable Crops Novi Sad

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Dry bean (*Phaseolus vulgaris* L.) is one of the most important food legumes and source of proteins and dietary fibers. Two major gene pools exist in cultivated dry bean, one being Middle American, and second Andean. Dry bean accessions and cultivars belonging to each of these two gene pools are distinguished based of phenotypic traits, as well as on molecular and biochemical level. Most of the dry bean germplasm is maintained *ex situ* in gene banks and collections of the research institutes around the world which is characterized with high level of genetic diversity. Possibility to identify this genetic variation is of greatest importance for conservation and utilization of dry bean germplasm. Phenotypic evaluation is the first step in discrimination and classification of these accessions.

The aim of this work was to assess phenotypic diversity of 37 dry bean genotypes (domestic and foreign cultivars and landraces) held at dry bean breeding collection of Institute of Field and Vegetable Crops, Novi Sad. We analyzed eight qualitative traits (growth type, color of flower standard, color of flower wings, pod color, seed pattern, color of seed coat, seed brilliance, seed shape) following the Bioversity International Key descriptor for common bean and six quantitative traits (number of pods per plant, number of seeds per plant, seed weight per plant, 1000 seed mass, number of days to flowering, number of days to maturity). Multivariate analyses, consisting of principal component analysis and cluster analysis were applied. In a principal component analysis first two axis explained 46.9% of variation, and the most important traits in discriminating between genotypes were separated. The genotypes differed mostly for number of pods per plant, number of seeds per plant, color of flower standard, color of flower wings, seed coat color and 1000 seed mass. Positive correlations were observed between number of pods per plant, number of seeds per plant, seed weight per plant and growth habit, as well as between growth habit and number of day to flowering and number of days to maturity. Negative correlation was observed between 1000 seed mass and other yield components and growth habit. Both principal component analysis (PCA) and cluster analysis clustered the genotypes into two major groups presenting Mesoamerican and Andean gene pools. Only two foreign cultivars (KP-12 and KP-13) were distinguished as third, separate group. Possible hybrids between these two genepools were identified, meaning genotypes with a combination of traits representing Andean or Mesoamerican gene pool.

Key words: *Phaseolus vulgaris*, phenotypic variation, gene pool

Relationship between NDVI and grain yield at different growing stages in winter wheat

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Grain yield represents one of the most important traits in wheat breeding programs and large-scale production. For early estimations of green biomass, chlorophyll content, leaf area index and grain yield at canopy level, different rapid and nondestructive methods such as NDVI are commonly used. The main aim of this work was to determine optimal period for grain yield estimation in winter wheat by use of NDVI. The experiment with 29 wheat cultivars was conducted at experimental field of Institute of Field and Vegetable Crops, Novi Sad. The NDVI readings were taken at the end of the tillering and at heading stage. Results from this study show variation in grain yield and NDVI values measured at both growing stages. The correlations between yield and the NDVI values were positive. Stronger correlation with grain yield was observed at end of tillering ($r=0.67$), in comparison to the heading stage ($r=0.56$). Therefore, in wheat management, reliable biomass and grain yield estimations during early growing stages, when crop yield may still be improved in response to increased availability of N fertilizers, may allow decisions to be made regarding the need for additional inputs.

Plant phenotyping installations available for EPPN2020 transnational access at the VIB-UGent Center for Plant Systems Biology, Belgium

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The VIB-UGent Center for Plant Systems Biology has three WIWAMs ('weighing, imaging and watering machines') available for transnational access (TNA) within EPPN2020. All three of them are dedicated to soil-grown plants, either in growth rooms (WIWAM_Arabidopsis and WIWAM_maize) or the greenhouse (PHENOVISION). They include automated systems for plant transport to stations for controlled irrigation with water or other solutions, and dedicated platform areas for imaging. All installations provide RGB imaging, whereas the greenhouse system also includes thermal and hyperspectral imaging systems. All WIWAMs are connected to an in-house developed data management system called PIPPA ('PSB Interface for Plant Phenotype Analysis'), including a central database, web interface for experimental setup and data analysis, and server storage. Besides measurement of traits based on image analysis, destructive sampling procedures for organ and cellular level analyses of growth have been established. Current data analyses pipelines are focused on Arabidopsis and maize. TNA users will receive training and support in data analysis for these species. For other species, advice can be given based on experience in Arabidopsis and maize.

Evaluation of hyperspectral data for assessing the physiological traits of diverse wheat genetic resources in field phenotyping

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The hyperspectral analysis represents one of the most promising tools for application in field phenotyping, with potential to provide complex information on different properties of crop canopies. However, the open question is the reliability of hyperspectral indices in the estimation of leaf properties when applied in a broad spectrum of genotypes differing in plant and leaf morphology, anatomy and chemical composition of leaves. To examine this issue, we tested the set of wheat genebank accessions with a broad phenotypic diversity using the hyperspectral field records as well as the subsequent analyses of phenotypic and physiological traits, such as leaf area, leaf thickness (measured as leaf mass per area unit, LMA), leaf nitrogen content, chlorophyll and carotenoid content, chlorophyll a to b ratio, chlorophyll to carotenoid ratio, SPAD value, etc. We found relatively high diversity in all observed traits (thick vs. thin leaves, high vs. low chlorophyll concentration; very small vs. very large leaves), providing good background for correlation analyses between the hyperspectral parameters and related phenotypic traits. We found that the parameters proposed in literature for estimation of some traits are not useful to be used for germplasm with a large or unknown phenotypic variability. Anyway, we found a few parameters correlating well across the entire collection of wheat genotypes, which can be regarded as more reliable and universal, useful for the use in phenotyping in genebank wheat collections or wheat breeding. Our results can be useful as a background for the next activities in phenotyping for biomass improvements, nutrient use efficiency or abiotic stress tolerance in wheat and other crops.

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Can UAV's help to make variety testing and breeding more objective and more efficient?

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Today breeding and variety testing are becoming more and more important due to a faster changing environment. Therefore breeding of new varieties and the testing process of new varieties should be efficient and objective. However, breeder or technician often subconsciously influences these processes. Phenotyping and in particular the use of unmanned aerial vehicles (UAV's) in field trials might be helpful to increase the efficiency and objectivity of breeding and variety testing. Furthermore, collecting data with UAV's can open up new possibilities to analyze plant parameters that are barely recognizable by eye as for example nitrogen uptake or plant development.

The Swiss federal center of agricultural research (Agroscope) is responsible for variety testing of different crops. In 2015, a project using drones with maize genotypes started to complement data conventionally investigated by eye with data collected by a fixed-wing UAV containing a red-green-blue-camera and a red-green-near infrared(NIR)-camera. The aim of this project is to identify opportunities to replace time-consuming parameters by indexes obtained from UAVs and to evaluate benefits and difficulties and limits of its use.

Flights were conducted in intervals of two week in maize trials at several locations in Switzerland. NDVI (normalized difference vegetation index, defined as $(NIR-red)/(NIR+red)$), canopy cover and plant height were obtained in each flight. Datasets were correlated with conventionally obtained data. Preliminary results show high correlations between canopy cover and NDVI (UAV) with early vigor (rating by eye). The use of an UAV seems to have potential to replace time consuming parameter investigation and therefore offers the possibility to make variety testing and breeding more objective and more efficient. The use of a fixed wing UAV has the benefit, that a big area can be covered with one flight. On the other hand, for some parameters the resolution of the images is not good enough due to the minimum operating altitude of 40 m.

Assessment of sugar beet hybrids based on morphological and root quality traits

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Sugar beet is the most important plant for sugar production in Europe and other temperate climate regions of the world. Broad genetic diversity is crucial for successful breeding program. The main objective of this study was to evaluate 10 sugar beet hybrids based on their morphological and root quality traits and to determine which genotypes are preferable source for new gene collection and selection program. The trial was performed during the growing season of 2016 on the fields of the Institute of field and vegetable crops, at location Rimski Šančevi. Among the explored genotypes, two were from domestic breeding centers while eight belonged to foreign seed institutions. Standardized mean values of all characteristics were used for analysis. The evaluation included Principal Component Analysis (PCA) for the following traits: root weight (g), root length (cm), root diameter (cm), root volume (cm³), root/head ratio (%), specific root weight (g/cm³) and sugar content (%). According to results significant level of diversity was found for all examined traits. Four principal components with their values of 50,2%; 24,4%; 11,9% and 10,4% determined total variance by the amount of 97,96%. Investigated genotypes could be divided into four distinct groups.

Keywords: sugar beet, root, PCA, multivariate analysis

Evaluation of fatty acids and tocopherols content in NS rapeseed collection

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The focus in rapeseed breeding has turned to improving and altering the content and composition of salutary oil constituents, such as oleic acid and linolenic acid contents and tocopherols. The objectives of this study were to exploit the variability of fatty acids and tocopherols content within the NS rapeseed collection, to classify the genotypes based on their oil quality and to identify genotypes with desired fatty acids and tocopherols content. A total of 49 genotypes were analysed for alpha and gamma tocopherols and oleic, linoleic, linolenic, stearic, palmitic, arachidic, behenic, eicosanoic, lignoceric and erucic acid content. Classification of rapeseed germplasm was performed using multivariate statistical methods. Principal component analysis, cluster analysis and two-way cluster analysis were applied. Principal Component Analysis revealed 5 components with Eigen value >1, which explained 78.70% of the total variability. Even so, the PC analysis identified oil quality traits that contributed most to the variation of analysed genotypes and can be used for facilitating the selection of desirable characteristics in rapeseed breeding. Cluster analysis and two-way cluster analysis helped identify genotypes with similar fatty acid and tocopherol composition. Two main groups could be identified on the dendrogram, the first having two genotypes and the second comprising 44 genotypes. Three genotypes did not belong to any group. The applied techniques can be helpful for identification, selection and optimized exploitation of rapeseed genotypes with desirable oil quality. No correlations between α - and γ - tocopherols or between tocopherol and oil content were detected. Individual tocopherols can be increased independently of each other and without affecting other major quality traits.

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Sunflower attractiveness to pollinators

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Yield of sunflower, as an open pollinated entomophilous plant, depends directly on the insect visitation. It has been found that various genotypes have a different degree of attractiveness for bees as the most common pollinators. The aim of this research was to examine the production of nectar and pollinator visits under the influence of different sowing densities as an important part of agricultural practice for achieving high yields.

A three-year trial with six different sowing densities (20,000 to 70,000 plants per hectare) and a total of 13 hybrids produced by IFVCNS was organized. The nectar yield was determined by examining 20 disk florets on three inflorescences per basic plot, using a micro-capillary method. The presence of pollinators was evaluated for three consecutive days during full flowering.

Although the amount of nectar at the density of 60,000 plants per ha was significantly lower than at 30,000, twice as many plants resulted in a significantly higher total yield of nectar per hectare. Regarding the pollinator's visit, the bees dominated. The highest pollinator numbers were recorded at smaller densities of 20,000 and 30,000 plants per ha, while for other sowing densities there was no significant difference in the visit. The most attractive hybrid had twice more visits than the least attractive.

The sowing density significantly influenced the pollinator's visit, but only in the plots with twice as smaller number of plants per hectare. Based on the obtained results, the melliferous value of a hybrid and its attractiveness should be assessed taking into account not only the nectar quantity, but also the agroecological conditions at the site, and the presence and condition of pollinators. Phenotyping in controlled conditions with a precise analysis of additional factors including scent could provide more useful results and advance this field of research.

Key words: sunflower, pollinator visit, nectar, sowing density

Fruit phenotypic evaluation of F₂ sweet pepper progeny from Amfora x Piquillo

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Peppers (*Capsicum annuum* L.) have a very diverse use in Serbia. Preparing ajvar is the most common way in Serbia to preserve pepper for wintertime. Besides, to larger fruits, high dry matter content in red pepper fruit is very important for better ajvar. The goal of our breeding work is to get large fruits with a high dry matter content. For this purpose, we crossed Amfora (kapia variety from the Institute of Field and Vegetable Crops, Novi Sad, Serbia) and Piquillo de Lodosa (kapia variety from Spain) in 2014. The next year 2015, F₁ generation were produced to get F₂ seed. In 2016, one hundred and eighty F₂ plants were planted in 10 rows. Also parent plants (Amfora and Piquillo) were planted. Distance between plants was 70 x 25 cm. For this purpose, we randomly chose 18 F₂ plants and covered with agro textile isolators. All fruits in physiological maturity were harvested in October and used for a phenotypic evaluation. The fruits were evaluated for seven quantitative traits: weight, length, diameter, index, locule number, pericarp thickness and total soluble solids (Brix). The principal component analysis (PCA) was used to identify the most significant traits and to found the difference between the plants. Software package Statistica ver. 12, was used to evaluate the level of diversity for pepper fruits and to rank the contribution of the variables. Mean values per genotype were standardized and used for analysis. According to PCA, Brix had a positive influence in the first principal component (PC1), while fruit weight, fruit diameter and pericarp thickness had a great, but negative influence. Fruit length and index had the most important and negative correlation in PC2. Fruits from F₂ plants were between parents (Amfora and Piquillo). Fruits from plants 15 and 5 tended to be more like Amfora (heavier fruits), while fruits from plants 7, 10 and 13 had a higher Brix like Piquillo. The most distinguished fruits had plant 17 because the highest fruit index.

Interaction response of high yielding NS hybrids of sunflower

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Adaptability and stability evaluation of genotypes involved in commercial production in divergent agroecological environments is of great importance in order to obtain high and stable yields. The aim of this research was to evaluate the interaction response, i.e. adaptability and stability, of high yielding commercial hybrids of sunflower. Research included 13 commercial sunflower hybrids developed at the Institute of Field and Vegetable Crops from Novi Sad. Hybrids were evaluated during 6 years across 19 localities in Serbia. The Additive Main effects and Multiplicative Interaction (AMMI) model was used to evaluate genotype x environment interaction. According to average yields the most favourable year for production was 2016 with average yield of 3.21 t/ha. Accompanying this result according to climatic data year 2016 was the most favourable for sunflower production. Analysis showed that in total average during 6 years the best producing sunflower hybrids were Ns Fantazija, NS Konstantin, NS Oskar, NS Romeo and Dusko with average yields over 3,90 t/ha. AMMI analysis revealed that expression of hybrids was mainly influenced by environment. In terms of stability and adaptability, during six years period, AMMI analysis showed that hybrids with the best stability, i.e. the lowest scores of interaction were NS Fantazija and NS Romeo. Calculated AMMI stability value (ASV) revealed that all NS sunflower hybrids were exceptional in terms of yield stability during six years period because values in all hybrids were lower than 2 which are considered highly stable. Taking into account that NS sunflower hybrids were grown on 19 localities during 6 years result of this research showed that they are well adapted to divergent agroecological environments with the ability to produce high yields.

Key words: adaptability, stability, sunflower, interaction, environments

The use of NIT technology for determination of oil content in oilseed

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Phenotyping of numerous genotypes for different quality traits in plant breeding program often requires fast, reliable and non-destructive analytical techniques. Near Infrared transmittance (NIT) technology is one of the widely used techniques. It possesses the following benefits: high performance scanning spectroscopy for robustness, consistency and small quantity of sample (about 50 g) in comparison to cereals (about 300 g). Actual properties of the device depend upon production region, applied reference method, year of harvest and weather conditions. Therefore, the aim of the study was to examine possible advantages of using NIT technology for determination of oil content in oilseed samples in comparison to commonly used techniques.

The oil content of forty different samples of oilseed (36 samples of rapeseed, 3 samples of camelina and 1 sample of mustard seed) was determined by standard Soxhlet extraction method and Bruker minispec 20 mq Nuclear Magnetic Resonance (NMR) device. Furthermore, this quality trait was also determined by whole grain analyser using NIT technology. This device used Artificial Neural Network calibration curves developed on 5000 number of samples for rapeseed.

The results of the study showed that the correlations among commonly used techniques and NIT technology were positive and statistically significant. The highest correlation ($r=0.9976$) was gained between standard Soxhlet extraction method and Bruker NMR device. Furthermore, correlation between NIT technology and Bruker NMR device was $r=0.9721$ while the lowest correlation ($r=0.9644$) was observed between NIT technology and Soxhlet extraction method. From results it can be concluded that NIT technology can be successfully used as a common technique in oilseed breeding programs especially if suitable calibration curves for every oilseed cultivar are developed.

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Cytological analysis of *Camelina sativa*, *Brassica nigra* and *Sinapis alba* from a germplasm collection in Novi Sad

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High quality oils and other traits desirable in organically produced food, brought species like *Camelina sativa*, *Brassica nigra* and *Sinapis alba* back to the farmer's fields. Their re-introduction also resulted in broadening of IFVCNS Brassicaceae germplasm collection with accessions of those species. The collected accessions were investigated for flower morphology, pollen features and number of chromosomes. Flower morphology of camelina and mustard were analysed microscopically. Pollen viability was determined using the Alexander staining method, while size and shape of pollen grains was determined with the help of AxioVision LE software. The number of chromosomes was counted in meiosis using aceto-carmin method. Fertile pollen grains were rounded with a thin exine and three visible septa in all tested species. In three camelina accessions average diameter of fertile pollen grains amounted to 23.84 µm, 23.82 µm and 24.19 µm, respectively, while sterile grains were smaller with 19.52 µm, 19.08 µm and 21.46 µm. Pollen of mustard had a larger diameter with 28.93 to 29.84 µm for fertile, and 21.95 to 22.89 µm for sterile pollen grains. Pollen viability of 12 analyzed accessions was high and ranged from 91.35% (Camelina 50) to 99.20% (Camelina 49). Lower pollen viability was only found in one sample of black mustard (69.23%). Accession Camelina 39 had meiocytes with 8, 16 and 20 bivalents in diakinesis and metaphase I supporting a chromosome number of $2n=40$, while black mustard accession SO1 had 16 and 20 chromosomes. White mustard was more difficult for chromosome number determination, but in accession 43 several anaphase meiocytes were found with 8 chromosomes on each cell pole and a total of 16 chromosomes.

Key words: camelina, black mustard, white mustard, pollen, chromosomes

This work was funded by the Serbian Ministry of Education, Science and Technological Development (project TR31025) and Serbian-Austrian bilateral cooperation project 451-03-01039/2015-09/42.

Duration of pre-heading period and its relationship with some grain properties in wheat genotypes

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Although grain yield in wheat is more correlated with grain number per unit area, high and stable yields are often positively associated with increased grain weight. Time of heading and duration of pre-heading developmental phases have been recognized as important traits that allow adjustment of crop phenology with the available resources for grain growth. Therefore, main objectives of this study were to analyze duration of pre-heading period and estimate its relationship with grain properties (test weight and 1000 grain weight) in wheat genotypes. For this study 16 divergent genotypes of wheat were selected. Trial was conducted during the growing season 2015/2016 at the Experimental Field of the Institute of Field and Vegetable Crops, Novi Sad. Main plot was 2 m² and randomized block design with three replications was applied.

Mean values of the analyzed traits showed that the average pre-heading period (number of days calculated from 1 January to heading date) was 127.4 for all genotypes. Genotypes 1 and 16 had the lowest value for pre-heading period (118), while genotypes 4 and 9 had the highest value (140). The correlation is one of the most common and most useful statistics that describes the degree of relationship between two variables. Further, correlation studies among yield contributing traits may help in indirect selection of yield components. Our results showed presence of significant correlations between studied traits. Between duration of pre-heading period and test weight significant ($p < 0.05$) negative correlation was determined ($r = -0.544$), while correlation between duration of pre-heading period and 1000 grain weight was highly significant ($p < 0.01$) and negative ($r = -0.608$). Positive and highly significant ($p < 0.01$) correlation was determined between 1000 grain weight and test weight ($r = 0.775$).

Based on the results of this research, it can be concluded that significant relationship existed between analyzed traits. Duration of pre-heading period was in significant and negative correlation with both, test weight and 1000 grain weight. Therefore, increase of pre-heading period duration should not be an appropriate strategy for high grain weight achievement, but development of early and medium early cultivars should be an adequate strategy for grain yield improvement in wheat via increasement of grain weight in this agro-climatic area.

Key words: *wheat, genotype, correlation, grain properties*

White mustard (*Sinapis alba* L.) agronomical characteristics variability

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Sinapis alba L. is an annual plant of the family Brassicaceae. VIR collection of *S. alba* consists of 272 accessions of different geographical origin. *Sinapis alba* is a cross-pollinated crop. Major half of the collection consists of the accessions collected in Russian Federation and the other half from European countries. White mustard occupies minor crop area in Russian Federation, it is cultivated as oilseed, forage and honey crop. *S. alba* accessions are annually regenerated, maintained and studied in the fields of the Institute's branches and experimental stations. Comprehensive study of *S. alba* in the conditions of North-West region of Russian Federation showed the vegetation period variation from 71 to 107 days, plant height varies from 75 to 140 cm, the weight of the seeds collected from 1m² is 290 g, weight of the seeds from one plant varies from 2,7 to 10,1 g. *S. alba* oil content amounts 34%, protein 25,4%. Fatty acid composition is characterized by palmitic acid content C16:0 (2,8%), stearic acid C18:0 (1,3%), oleic acid C18:1 (32,4%), linoleic acid 18:2 (8,9%), linolenic acid C18:3 (9,6%), icosenic acid C20:1 (11,5%), erucic acid C22:1 (35,2%). The press cake contains 35% protein and 12% oil.

PCA analysis of yield components of onion (*Allium cepa* L.)

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Agricultural practice of growing vegetables focuses primarily on the effect of genotype which limits the cultivar yield potential. Environmental factors and cultivation practices further determine the degree of the said potential. One of the key goals of breeding programs is increased yield potential. Onion bulb yield depends on many components and it is important to understand the relation of yield with its components, which should be balanced with environmental conditions. This assures the choice of genotypes with the highest number of favourable traits and high yields. The trial was carried out at the experimental field of the Institute for Vegetable Crops in Smederevska Palanka in Serbia as a randomized block design in five replicates. Total of 24 genotypes from the Institute's onion germplasm collection were tested. The following were determined in onion genotypes: total bulb yield, diameter, height, index, weight, total dry matter content and growing season. PCA was used to calculate and group the data for 24 onion genotypes and seven analysed traits. Two components explained 70.86% of the total variability. According to the correlation matrix, bulb yield was most strongly correlated to bulb weight and diameter. The genotypes IFVC-AC 21 and IFVC-AC25 had the highest effect on bulb yield increase, and genotype Tetenji rubin had the highest effect on bulb yield decrease. Out of the analysed traits, only dry matter was negatively correlated with yield and other yield components.

Raman microscopy/spectroscopy: non-destructive tool for the characterization of tomato fruit quality

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Raman microscopy/spectroscopy is a method for non-destructive qualitative and quantitative sample analysis and have recently been used for analysing of compounds in plant materials. The aim of presenting study was to investigate potential of Raman spectroscopy in the tomato fruit quality characterization, with special attention to carotenoids and vitamin C analysis.

An experiment was conducted in plant growth chamber in controlled conditions at the Faculty of Agriculture, University of Belgrade. Fruits of wild-type tomato Ailsa Craig and its mutant *flacca* were harvested in red-ripe stage and sliced with microtome blades. Slices were analysed by Raman spectroscopy (Raman Horiba XploRA), with a laser excitation lines 532 and 785 nm and 1-5 s integration time. The filter was set to 100 % and we used 100 x LWD.

The results indicated that Raman spectroscopy has the great potential for fast, non-destructive analysis of fruit quality, especially of carotenoid content. Analysis of carotenoids resulted in several strong peaks which could be also used for screening different tomato genotypes both in optimal and stress conditions. Thus, Raman spectroscopy could be a useful tool for fruit phenotyping.

PCA Classification of tomato genotypes based on physical and chemical fruit characteristics

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Importance of tomato, being considered as "functional food" is reflected by the rising trend of harvested areas and consumption *per capita*, in recent decades. Beside specific demands, fruit quality is common for both, consumers of fresh fruits and processing industry. The objective of this study was to characterize 20 tomato genotypes based on physical and chemical quality characteristics and to segregate perspective genotypes for improvement of tomato quality by breeding programs. The experiment was carried out during three consecutive years (2010-2012) at experimental fields at Rimski Šančevi site, near Novi Sad. Five landraces, four old varieties, eight breeding lines and three commercial cultivars were chosen for the investigation. Following fruit characteristics were analyzed: average weight (g), length (cm), width (cm), pericarp thickness (mm), locule number, moisture content (%), total soluble solids (°Brix), ash content (%), total acidity (%) and pH value. Diversity of genotypes in all analyzed traits was found. Four principal components explained 90,6% of total variance or 36,5%, 24,2%, 19,8% and 10,1, respectively. Along the axis of the first main component, genotypes were classified into three groups. Genotypes with the thickest pericarp, highest total soluble solids, ash content, and acidity were identified, as promising for quality improvement in tomato breeding programs.

Keywords: fruit, PCA, tomato, quality

Yield Stability and Selection Strategies for Chickpea in Portugal

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Chickpea (*Cicer arietinum* L.) is a very important pulse crop in the world and is mainly grown for human consumption, providing an important source of protein. It is cultivated on a wide range of environments and traditionally sown during March. It is extremely affected by long periods of drought during the reproductive phase and by high temperatures, which limit production seriously. As a consequence, there is a need to develop chickpea germplasm with resistance to abiotic stresses mainly terminal drought and heat. The present investigation was carried out to study stability for seed yield and its components in genetically diverse genotypes of chickpea. Trials were conducted during three years under rainfed conditions in Elvas, Portugal. Genotype x environment interaction (GE) was observed in all experiments. Cross-over interaction between top-yielding and low yielding cultivars occurred across the environments. In general, genotypes that fasten their development cycle showed higher grain yield, especially in dry years. GE interaction analyses were calculated by regressing genotype means against a site index, the mean yield or the mean site effect. The analysis of results for the three years showed four groups of genotypes: 1) genotypes with high adaptation to distinct environments; 2) genotypes adapted to favourable conditions, but with bad performance under adverse conditions; 3) genotypes with very good adaptation for poor environments and 4) genotypes with no adaptation. From this experiment, we are able to select two genotypes that stand out for their high performance in all seasons and introduce them into the national chickpea breeding program.

Phenotyping and genomic selection on advanced breeding lines of bread wheat

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The main challenge in agriculture for the next decades is the sustainable increase of plant crops yields. It is urgent to sustainably increase production using higher yield crops. To reach this goal it will not be enough to pursue conventional breeding strategies, we need to introduce molecular genetics tools which will allow to accelerate the breeding process, shortening the number of years involved in breeding programs for obtaining new and more well-adapted varieties in a world in permanent climatic, demographic and social change, but responding to environmental and market demands.

Genomic selection (GS) is a novel approach to increase selection gain in crop and livestock breeding. GS uses multiple genomic molecular markers to predict the effects of complex, quantitative traits in animal and plant breeding by modeling those effects and predicting the sum of all of them. A goal for GS is to produce a genomic estimated breeding value (GEBV), from which candidates can be selected by genotyping before phenotypic evaluation. The GS modeling approach is therefore an innovating strategy that makes use of phenotypes for characterization of specific traits and genotypes for prediction of those traits, shortening the period of development of new improved lines or varieties in breeding programs. Conventional breeding of grain crops continues to deliver improved varieties to farmers with little evidence of a leveling in yield. In most breeding programs, there is almost no direct selection for interest traits, such yield and grain quality apart from some phenological parameters like heading and maturity date, growth cycle, plant height and tolerance/susceptibility to main diseases.

Phenological traits are governed by a few genes of large effect. In early generations, breeders rely on their experienced eye to select for what are considered to be appropriate plant types, and in later generations rely on yield testing and quality parameters, with a polygenic behavior. We will present phenotyping results of advanced wheat lines under GBS (genotyping by sequencing) evaluation. Selection on single or limited numbers of markers for quantitative traits often misses a substantial portion of the genetic variance contributed by loci of small effects. Genomic selection modeling therefore takes advantage of the increasing abundance of molecular markers such GBS through modeling of many genetic loci with small effects.

Improvement of phenomics methods should contribute to increase accuracy on prediction models of estimated breeding values, alone or together with molecular markers. Although we are using only conventional methods for phenotyping, discussing the new approaches and technologies is an important issue.

Diversity of confectionery sunflower based on morphological characteristics

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Around 10% of the world annual production of sunflower seed is used for non-oil purposes, for instance it is used mainly for confectionery and snack food, as well as for bird and pet food. Breeding of confectionery sunflower is directed by different markets demands regarding the seed size, hull color and other traits, which makes this process more difficult and costly. Knowledge of the amount and distribution of genetic diversity within the cultivated sunflower germplasm is of great value for ongoing crop improvement efforts. Land races and OP varieties have huge genetic variation and are well adapted to local soil types and climatic conditions, as well as other environmental factors. They are the source of many desirable genes, especially those addressing higher adaptability to environmental conditions and resistance to certain diseases. However, little is known about the levels and distribution of genetic variation within the confectionery sunflower gene pool. Morphological characterization can serve as a good starting point to increase the knowledge of genetic diversity as the basic prerequisite of a plant breeding program. Confectionery sunflower varieties will be used in this study were sampled from Hungary, Russia, Turkey, China, Iran and Serbia. Female inbred lines, male inbred lines and hybrids were developed at the IFVCNS. Therefore, the aim of this study will be: determine the extent of phenotypic diversity in confectionery sunflower based on morphological traits, and determine the possibility for use of the studied material in confectionery sunflower breeding program. The primary objective of this research will be determining the extent of phenotypic diversity. It's expected the high diversity index value a confectionary sunflower germplasm collection. It is expected that the seed main color, shape of distal part compared to insertion of petiole, stripes on margin, stripes between margins could be useful traits for genotype discrimination.

Dissecting genotype x environment interactions for yield and quality in oats: integration of ground, UAV and satellite data

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National Institute of Agricultural Botany (NIAB)

The goal of the multi-partner, industry-led 'OptiOat' project, funded in part by InnovateUK, is to improve the understanding of the physiology of yield formation and determination of grain quality in oats for human consumption. To match the growing demand for healthy breakfast and snack food products, and the specific requirements for milling and nutritional characteristics, the interactions between varieties, farm inputs and the growing environment need to be understood. For the first time, this knowledge will be packaged in a Growth Guide and online decision support tool (driven by a novel oat growth model) to help guide growers to identify which variety characters are critical for optimising yield and quality. The project combines detailed, time-series ground measurements with satellite and UAV measurements to develop and validate algorithms for translating image and spectral sensor data into quantifiable crop parameters to enable growers to optimise in-season management for yield and quality across fields.

Data have been gathered from Reference crops of two winter varieties (Mascani and semi-dwarf Balado) and one spring variety (Canyon), sown in five geographically distinct locations throughout the UK over four seasons. Detailed measurements were made from sowing to harvest of crop growth and development, yield components, grain quality, soil and climatic conditions. UAV-based measurements were done at four key growth stages to quantify canopy dynamics, crop height and estimate biomass. In parallel, commercial crops of Mascani and Canyon were similarly monitored across key oat growing regions in the UK to generate datasets from over 100 sites across 4 years.

Project findings should provide a novel resource for advancing the understanding of oats for growers, breeders and millers.

OptiOat is led by PepsiCo (Quaker Oat brand), in collaboration with NIAB, ADAS, Aberystwyth University, the James Hutton Institute and Environment Systems.

Session 2: Phenotyping/Breeding for nutrient efficiency

Oral presentations:

The impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilisation

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The process of domestication has led to dramatic morphological and physiological changes in crop species due to adaptation to cultivation and to the needs of farmers. To investigate the phenotypic architecture of shoot- and root-related traits and quantify the impact of primary domestication and secondary domestication, we examined a collection of 36 wheat genotypes under optimal and nitrogen-starved conditions. These represented three taxa that correspond to key steps in the recent evolution of tetraploid wheat (i.e., wild emmer, emmer, and durum wheat). Overall, nitrogen starvation reduced shoot growth of all genotypes, while it induced the opposite effect on root traits, quantified using the automated phenotyping platform GROWSCREEN-Rhizo. We observed an overall increase in the entire shoot and root growth traits from wild emmer to durum wheat, while emmer was generally very similar to wild emmer, but intermediate between these two subspecies. While the differences in phenotypic diversity due to the effects of primary domestication were not significant, the secondary domestication transition from emmer to durum wheat was marked by a large and significant decrease in the coefficient of additive genetic variation. In particular, this reduction was very strong under the optimal condition, and less intense under nitrogen starvation. Moreover, while under the optimal condition both root and shoot traits showed significantly reduced diversity due to secondary domestication, under nitrogen starvation the reduced diversity was significant only for shoot traits. Overall, a considerable amount of phenotypic variation was observed in wild emmer and emmer which could be exploited for the development of pre-breeding strategies.

Integrated analysis of plant growth and development using high throughput multi-sensor platforms at IPK

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Plant phenomics describes automated and high throughput amenable techniques that allow for quantitative monitoring of large plant populations with respect to breeding-relevant traits such as higher yield or biomass accumulation, plant resistance to biotic and abiotic stresses as well as plant nutrient use efficiency. Currently, the IPK runs three plant phenotyping facilities for high-throughput imaging of whole plants of small size (such as *Arabidopsis*), medium size (such as barley) and large size (such as maize, Junker et al. 2015). In each of these phenotyping systems plant images are acquired in near-infrared (NIR) and visible spectra (for RGB and fluorescence imaging) from top and side views. Imaging is performed repeatedly and thus enables to follow plant growth dynamics over development. The Image Analysis Platform (IAP, Klukas et al. 2014) automatically extracts plant architectural traits (such as plant height and width, projected leaf area, estimated volume), colour-related and physiological traits. The existing high throughput plant phenotyping facilities have been extended for the analysis functional chlorophyll fluorescence using pulsed amplitude-modulated chlorophyll fluorescence imaging systems (Tschiersch et al. 2017) and for the acquisition of 3D height profiles of plants and plant stands. These systems are integrated into the plant phenotyping procedures and thereby allow for the simultaneous acquisition of multiple complementary plant traits. Based on a summary of phenotyping experiments performed within the last years the utility of the facilities for the investigation of various biological questions is presented. Furthermore recent achievements in phenotype data management, standardized metadata representation (MIAPPE), and data publication will be introduced.

High throughput root phenotyping using the “Rhizo” suite

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While automated high throughput plant shoot phenotyping (*e.g.* measurement of leaf, fruits) is progressing quite rapidly in a range of platforms, morphometric characterization of plant roots (root architectural traits) is still lagging behind, due to both the high plasticity of roots and obvious technical difficulties to access them in situ. This is especially relevant for field experiments but also in controlled conditions.

In order to get automated, non-destructive and fast phenotyping of roots, a range of tools and methods have been conceived within the Phenotyping Platform for Plant and Microorganisms Interactions (4PMI) in the context of the Phenome Project (French Plant Phenotyping Network, <https://www.phenome-fppn.fr/>), and accessible in transnational access through the EPPN2020 project ([http://www.2020-horizon.com/EPPN-European-Plant-Phenotyping-Network\(EPPN\)-s211.html](http://www.2020-horizon.com/EPPN-European-Plant-Phenotyping-Network(EPPN)-s211.html)).

Specialized containers (RhizoTubes) and imaging cabins (RhizoCabs) have been developed and already evaluated for a range of species in different environmental conditions (Jeudy et al., 2016). This will be briefly summarized together with recent results conducted on large number of maize, wheat, and pea genotypes subjected to contrasted environmental conditions. How to access through EPPN2020 to these tools will be presented.

Alltogether, besides the bottleneck represented by image analysis, this will demonstrate the power of high throughput root phenotyping devices and methods to identify plant more tolerant to abiotic stress, including conditions of fluctuating conditions of soil resources availability.

Jeudy C, Adrian M, Baussard C, Bernard C, Bernaud E, Bourion V, Busset H, Cabrera-Bosquet L, Cointault F, Han S. et al. 2016. RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation. *Plant Methods* 12(1), 1.

Relationship between liposoluble fingerprints and botanical origin of various agricultural crops

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Cereal grains and their products are among the most important staple foods in human nutrition worldwide. However, it was scientifically proven that the substitution of white or whole-grain wheat flour with flour of other cereal and pseudocereal species may increase nutritional and/or functional value of the product, which could have a positive impact on human health if consumed regularly.

Samples of all analysed agricultural crops were obtained from the cultivated, living collection of the Institute of Field and Vegetable Crops, in Novi Sad, Serbia. All species were grown in the same year and on the same experimental field, thus enabling a comparison to be independent of differences in environmental conditions. The collected samples included various cultivars of different cereals and pseudocereals: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), triticale (*Triticosecale* Wittm.), corn (*Zea mays* L.), spelt (*Triticum spelta* L.), amaranth (*Amaranthus* L.), and buckwheat (*Fagopyrum esculentum* Moench).

Grain samples were ground to flour, lipid components were extracted using n-hexane and derivatized with a methanol solution of trimethylsulfonium hydroxide (TMSH). Extracted non-polar lipids were thus translated into volatile fatty acid methyl esters and further analysed on a gas chromatograph coupled to mass spectrometric detection (GC/MS). Peaks of lipids were separated from full-scan chromatograms using a specific fragmentation ion of 74 Da, the McLafferty rearrangement ion. Integrated surface areas of lipids detected on obtained chromatograms presented a fingerprint matrix subjected to a multivariate statistical data processing: cluster analysis and principal component analysis. Firstly, the samples of cereals (wheat and spelt) and pseudocereals (amaranth and buckwheat) were separated from each other using this method. Secondly, the samples of small grains (wheat, rye, triticale, barley and oat) were successfully separated from the analysed cultivars of corn. Considering different data acquisition methods on different analytical instruments used in the analysis of agricultural crops investigated in this study, a uniform binary system was applied to enable multivariate processing of all collected data. The results indicated a strong botanical separation between the analysed cultivars of corn, amaranth, buckwheat, spelt and small grains (wheat, rye, triticale, barley and oat).

Thus, it was shown that the proposed GC/MS liposoluble fingerprinting methodology is able to differentiate samples of various cereal and pseudocereal species, according to belonging botanical origin. This method could have a practical importance in the quality control procedures of grain flour and bakery products.

Reliable and efficient high-throughput phenotyping to accelerate genetic gains in Norwegian plant breeding

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New technologies like genomic selection and high-throughput phenotyping offer possibilities to increase genetic gains in plant breeding through more precise selection and shortening of the breeding cycle. However, considerable research is needed in terms of theoretical developments, statistical modeling and technical solutions to achieve this in practice. Here, we present the recently funded 4-year Norwegian project “Virtual phenomics” (vPheno, NFR 267806, 2017-2021), which is a collaboration between the plant breeding company Graminor, the Norwegian University of Life Sciences and world-leading groups in robotics, image analysis, statistical modeling and data management. In this project, we will develop novel statistical models to extract biologically relevant information from multispectral and hyperspectral images. The work will consist of developing reliable methods for capturing high-resolution images of field plots, and utilizing novel computational solutions to integrate top view images from drones with close-up images from robots to build 3D models that retain the original resolution and multispectral information. Computational algorithms will then be used to extract important physical and physiological traits that can be used directly as selection tools in plant breeding. By coupling multispectral data with grain yield and other direct measurements, statistical prediction models will be developed that plant breeders can use in early-generation selection to increase yield gains. User-friendly solutions will be developed through direct involvement of plant breeders in the project. By utilizing virtual reality technology, our ultimate goal is to “take the field to the breeder” and let the plant breeder observe the field plots and associated data through VR goggles.

In the 2016 and 2017 field seasons, field trials with a historical set of spring wheat cultivars grown at two nitrogen fertilization levels have been used to compare a Phantom drone and the Thorvald robotic platform as phenotyping tools. They were both fitted with a Micasense multispectral camera and images were taken repeatedly throughout the growth of the plants from seedling emergence to maturity. The image data from the two platforms were compared in their ability to estimate grain yield, plant height and other plant breeding related traits. Some preliminary results and experiences so far from this ongoing research will be presented.

Phenotyping soybean and common bean for better growth and nutrition under elevated CO₂

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Today climate changes are of major concern, and increasing atmospheric CO₂ (hCO₂) level is one of the most important and worldwide events. Currently the global CO₂ concentration is about 400 ppm, but it will rise to about 550 ppm by 2050. Elevated CO₂ affects plant growth, crop yield and nutritional status of agricultural products. Although hCO₂ has been previously associated with increased yields, recent studies found a significant negative effect in protein and mineral concentrations in several crops. Parallel to hCO₂, restricted soil Fe supply will also affect legume nutrition. On one hand, Fe has low solubility and on the other hand, about 30% of the arable land in the world is calcareous. The combination of these factors results in reduced Fe availability leading to reduced yields and possible lower nutritional quality. Here, we grew 18 soybean (*Glycine max*) and 18 common bean (*Phaseolus vulgaris*) cultivars under hCO₂ or ambient CO₂ with or without Fe restriction, and we conducted three experiments. The major goal was to harvest the grain and pod material and to look at the effect of hCO₂ on the nutritional composition (minerals, protein phytonutrients) of the different lines of both legume species, chasing genetic variability for the nutritional traits. We hypothesize that the nutritional variability will surely be due to several factors, amongst which changes in photosynthesis and respiration. Photosynthetic rate and morphological changes will indirectly affect sink-source relationships within the plant, and impact photo assimilate redistribution during grain fill. The first experiment (experiment 1) was a short-term study and it aimed at understanding the combined effect of iron deficiency and hCO₂. In the second and third studies the aim was to select cultivars with contrasting behaviors in the presence of different atmospheric CO₂ levels in growth chamber (experiment 2) or field (experiment 3) conditions. We phenotyped the cultivars of both species looking at biomass, nutrition, metabolomics and yield parameters. Preliminary data from these three experiments will be presented, including data generated in frame of a recently approved STSM. We believe that the combined information from these three studies conducted in growth chamber and field conditions will allow identifying the most CO₂-responsive genotypes and provide starting lines for future breeding programs.

We would like to acknowledge FCT (PTDC/AGR-PRO/3972/2014) and COST action FA1306

Poster presentations:

Root-microbe phenotyping in plant breeding: Current Knowledge and Future Needs

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Plant breeding during the past decades has been performed under high fertilization and intensive crop protection, leading to a substantial decrease of diversity among modern cultivars. The selection of genotypes under these conditions has also affected the ability of modern varieties to establish beneficial associations with soil functional microbial assemblages, like mycorrhizae fungi and symbiotic nitrogen fixing bacteria. The community structure of these microbial guilds is crucial for agricultural systems productivity since they are facilitating nutrient uptake and crop tolerance to abiotic stresses, like drought and salinity, while in some cases they are providing protection from soil borne pathogens.

During our plant-breeding program in Agricultural Research Institute, it was demonstrated that different genotypes of maize, cowpea and chickpea exploited differently the native microbial communities and this was associated with important plant traits like N uptake and yield.

Also, we found that the symbiotic nitrogen fixing bacterial community, identified in local cowpea and chickpea, was genotype-dependent while a significant interaction between nitrogen fixing bacteria and mycorrhizal inoculum has been noticed. Our results showed that genotypes with the most interesting plant traits were significantly correlated with associated functional microbial assemblages, like mycorrhizal fungi and nitrogen fixing bacteria.

The ultimate goal is to better understand how plant breeding process influence plant associated microbial functional communities and how these interactions can be used to select and improve important plant traits.

Biochemical parameters related to butternut squash fruit nutritional and sensory quality: a preliminary screening

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Butternut squash belongs to species *Cucurbita moschata* Duch. ex Poir. The fruits of the type are cream coloured, with or without very shallow grooves; small sized and weighted, mostly pear or club shaped ("bell type"). Owing to biologically active components from the orange fruit flesh, butternuts are considered delicious functional food, together with other *moschatas*. Therefore, maintaining good yields while improving nutritional and sensory qualities would be among the most important goals in breeding butternuts intended for cultivation in specific areas.

This study was aimed to explore the butternut squashes in terms of biochemical parameters (dry weight, sugar, protein, carotenoids, and cellulose percentage; pH and Brix) related to fruit nutritional and sensory quality. Since no single butternut variety originates from Serbian breeding centres, the accessions chosen for the analyses are foreign cultivars that are a part of *Cucurbita* collection of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The fruit samples were from field-grown plants, taken at full maturity stage and analysed by standard methods.

Although significantly variable among the accessions (except pH), all the parameters were within the range commonly reported for this squash type. Protein percentage was with the highest coefficient of variation, and pH with the lowest (24.4 and 3.6, respectively). Although moderate, the intervals of variation determined for sugar (0.7) and carotenoids (2.3) percentage provide the basis for breeding for improved fruit quality. Percentages of dry weight, sugar and carotenoids were positively correlated, and the fruits with higher values of the parameters generally had lower pH.

The results of this preliminary screening should be useful when selecting butternut squashes for crossings aimed to breed varieties with improved quality, adapted to the environments of Southeast Europe.

Phenotyping and accumulation of valuable nutrients in *Agrobacterium*-mediated genetic transformation system in common buckwheat

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Transformed hairy root culture in common buckwheat (*Fagopyrum esculentum* Moench) was investigated for accumulation amino acids and specific flavonoids. Transformation of *F. esculentum* hairy root lines induced by infected two types of explant, leaf and stem, with *Agrobacterium rhizogenes* was confirmed by PCR detection of *rol B* gene, and their capability to grow and accumulation of different amino acids and flavonoids was studied. Three lines from each explant types depending upon growth kinetics and phenotype were observed. The significant higher content of semi-essential amino acids such as lysine, isoleucine, valine, histidine and phenylalanine in selected hairy root lines from leaf and stem. Proline content in all selected hairy root lines significantly increased; it can be connected with biotic stress reaction occurred by interaction with *A. rhizogenes*. Increasing of sulfur-containing amino acids content in the different hairy root transformed lines from leaf and stems have been estimated. It was observed similar relationships of increasing of total flavonoids and flavons content together with antioxidant activity in selected hairy root lines extracts. 3 flavonoids were identified via HPLC analysis – rutin, hesperidine and kaempferol-3-rutinoside. It was observed decreasing of rutin content in selected hairy root lines what can evidence about rutin participation in defense signal to bacterial pathogens.

Chemometric discrimination of high- from low-lipid wheat cultivars using GC/MS data

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For thousands of years, wheat (*Triticum* spp.) has been among the most important staple foods in human nutrition, containing a large array of nutritionally beneficial micronutrients. Due to their often high per capita consumption, wheat products are an important dietary source of lipophilic antioxidants and vitamins, such as carotenoids, vitamin E, alkylresorcinols and steryl ferulates. Therefore, a lipophilic fraction of wheat kernels can be considered as an important factor influencing both human health and technology of wheat products. Various wheat samples were collected from the Institute of Field and Vegetable Crops in Novi Sad, Serbia, in order to develop a rapid method for an estimation of their lipid content. Analysed samples included winter varieties of a common wheat (*Triticum aestivum* L.): Renesansa (W1), Rapsodija (W2), Evropa 90 (W3), Pesma (W4) and Milijana (W5); spring varieties of a common wheat: Nataša (W6) and Venera (W7); and durum wheat varieties (*Triticum durum* L.): Durumko (W8) and NS Dur (W9). Wheat samples were ground using a laboratory mill, the non-polar lipid fractions were extracted using n-hexane, and derivatized using a methanol solution of trimethylsulfonium hydroxide (TMSH). Extracted triacylglycerols were thus transesterified into volatile fatty acid methyl esters. Analytical procedure was then conducted on a gas chromatography instrument coupled to mass spectrometric detection (GC-MS). The obtained GC-MS data in the form of full-scan chromatograms were acquired by Agilent MSD Productivity ChemStation software. Lipid compound identifications involved comparisons of the mass spectra with the Wiley 275 MS database using a probability-based matching algorithm (a match quality of 95% minimum was used as a criterion). Detected fatty acids in every analysed variety of wheat samples included methyl esters of: C14:0, C15:0, C16:1, C16:0, C17:0, C18:2, C18:1, C18:0, C20:1, C22:0, C23:0, C24:0 and C26:0. Surface areas of detected fatty acids were integrated and their numerical values subjected to a multivariate analytical procedure: hierarchical cluster analysis (HCA), using a Ward's method. High-lipid wheat samples (W2, W3, W4) were grouped on the left side of the obtained dendrogram, while low-lipid wheat samples (W1, W5, W6, W7, W8, W9) were grouped on the right. By performing a principal component analysis (PCA) of investigated fatty acids as variables, a PCA loadings plot was obtained. Variable groupings suggested that only three of them were important factors in discriminating investigated wheat varieties according to the lipid content: palmitic (C16:0), stearic (C18:0) and linoleic (C18:2) acid.

Thus, just by detecting and integrating the three suggested fatty acids it is possible to discriminate high- from low-lipid wheat varieties, aiming at the selection of wheats, being even richer in lipophilic nutrients than the current ones. Flours derived from such future cultivars may be promising ingredients for functional bakery goods and other cereal products.

Rutin content in seeds of European buckwheat (*Fagopyrum esculentum*) cultivars

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Buckwheat (*Fagopyrum esculentum* Moench) is an important functional food and rich source of vitamins, essential amino acids and phenolics, responsible for many of the health benefits and antioxidant properties. This pseudocereal contains quercetin-3-rutinoside or rutin as a major phenol in seeds, approx. 90.4% of total flavonols content. Rutin is an antioxidant that has many pharmacologically useful properties, such as anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective and vasoprotective effects. The aim of this study was to investigate content of rutin in seeds of cultivars from Western, Central Europe and Balkans: Serbia ('Novosadska'), Slovenia ('Darja', 'Prekmurska' and 'Cebelica'), Bosnia and Herzegovina ('Bosna 1' and 'Bosna 2'), Montenegro ('Godijevo' and 'Lokve'), Austria ('Bamby'), Czech Republic ('Ceska'), France ('La Harpe'), Slovakia ('Spacinska 1') grown in the Balkan area and to compare them with indigenous Balkan buckwheat cultivars. Rutin content was determined using HPLC-DAD analysis, on a Thermo Finnigan Surveyor HPLC system with a diode array detector at 350 nm. 'Bosna 1' (114.6 mg 100 g⁻¹ dry weight) and 'Bosna 2' (151.4 mg 100 g⁻¹ dry weight) were highlighted with the greatest rutin content, 13.6-34.7 and 18.0-45.9 times higher than in other cultivars, respectively. Beside these two cultivars, a scale made according to rutin content in buckwheat seeds organise investigated cultivars in the following order: 'Novosadska' > 'La Harpe' > 'Godijevo' > 'Darja' > 'Cebelica' > 'Bamby' > 'Ceska' > 'Spacinska 1' > 'Prekmurska' > 'Lokve'.

These results suggest that indigenous buckwheat cultivars contain important dietary antioxidants and could be of great interest for buckwheat breeders and an important source of functional food due to significant differences in their contents among cultivars.

Genotypic variation in zinc efficiency of Serbian maize hybrids evaluated in nutrient solution

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Differential Zn-efficiency (ZE) has been reported in many crops, including maize (*Zea mays* L.). Results on genetic variation in nutrient efficiency are of great importance for breeding strategies focused on crops biofortification. Experiment was conducted in nutrient solution with aim to study genotypic variation in ZE among nine Serbian maize hybrids. Plants were grown under controlled environmental conditions for four weeks under adequate (0.5 µM) and deficient (0.05 µM) Zn supply. ZE was calculated for the whole shoot dry weight as follows: $ZE (\%) = [0,05 \mu M / 0,5 \mu M] \times 100$. Hybrids NS 3022 and NS 4051 classified as Zn-inefficient had 2-fold lower ZE (47% and 45%, respectively) than Zn-efficient NS 6030 (96%). Interestingly, shoot/root ratios were depressed markedly due to Zn deficiency in all hybrids, irrespective of their ZE. Concentration and total content of Zn in leaves differed significantly between hybrids under both Zn treatments. Zn-efficient NS 6030 had 2-fold to 3-fold higher Zn concentration in leaves than Zn-inefficient hybrids. Results indicate to substantial variation in ZE efficiency within tested hybrids grown in nutrient solutions. Further investigation in field trials is needed for evaluation of Serbian maize hybrids in tolerance to Zn deficiency with aim to improve breeding of maize and agronomic practices related to micronutrients fertilization.

Session 3: Phenotyping/Breeding for perennial crops

Oral presentations:

An efficient phenotyping for selection in ancient grapevine varieties

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An efficient phenotyping of quantitative traits is always necessary in classical and modern plant breeding approaches.

Under the plant-breeder perspective, an efficient phenotyping must take into account two standpoints: (1) the availability of adequate tools to measure the target traits, (2) the rules that must be followed in order to ensure that the obtained data can be useful for selection purposes. Concerning first point, plant breeding benefits from simple, rapid and high-throughput phenotyping methods well adapted to the main agronomic and technological traits. Regarding the second point, plant breeding demands a well-planned phenotyping, which involves the establishment of field trials with efficient experimental designs and appropriate models for data analysis. This last concern arises because working with quantitative traits (the most economically important ones) requires the understanding of the meaning of the obtained phenotypic value. Concretely, it is necessary to know how much of the phenotypic variability is due to genotypic (e.g., heritable) causes and to evaluate the genetic gains of selection.

This work emphasizes the importance of an efficient phenotyping of quantitative traits for selection in ancient grapevine varieties. It is illustrated a methodology that already provided important outcomes: (1) phenotypic data for the most important traits (yield, quality traits of the must among others) from more than 170 field trials, and (2) tools for data analysis for increasing accuracy and precision of genotypic variance component estimate and predicted genotypic effects.

The applied methodology permitted to select polyclonal material from more than 60 ancient grapevine varieties, with prediction of high genetic gains for several traits, including yield gains ranging from 10 to 40%. In addition, it was possible to study the genotype by environment interaction for more than 120 grapevine clones. Furthermore, three detailed examples about the most useful experimental designs to phenotyping in grapevine selection field trials showed that the standard error of prediction of genotypic effects decreases between 4.6% and 7.7% when a resolvable row-column design is used instead of a randomized complete block design.

The current selection procedure applied to Portuguese grapevine ancient varieties affords important economic gains to vine and wine industry and is applicable to other ancient varieties of perennial vegetatively propagated crops in general containing high intravarietal genetic variability.

Phenotyping of perennial ryegrass by physiological fingerprinting and UAV remote sensing using RGB-, thermo- and multi-reflectance imaging

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The PhenoLab at the University of Copenhagen, available within the EPPN2020 network for transnational access, is a conveyor-band based automated high-throughput phenotyping platform in the greenhouse. It allows phenotyping of up to 117 13 x 13 cm or 468 8 x 8 cm pots by multireflectance, multifuorescence and thermoimaging. The pots can be individually randomized, rotated, measured and weighed. The soil water potential is measured continuously in each pot and the individual irrigation can be automatically adjusted accordingly, providing the possibility to maintain a certain soil water status and perform experiments at various drought levels within the same experiment. Whereas great advances have been made in the cost efficient and high throughput analyses of enetic information and non-invasive phenotyping, the large scale analyses of the underlying physiological mechanisms is lagging behind. However, the various scales of dynamic physiological responses need to be considered and genotyping and external phenotyping must be linked to the physiology at the cellular and tissue level. We have established an experimental platform for the semi-highthroughput analyses of activity signatures of key enzymes of carbohydrate metabolism and phytohormone profiles. This physiological fingerprinting approach has been used to analyse the physiological basis of the high fructan trait in different *Lolium perenne* cultivars. Fructan synthesis has been induced by cold treatment and in addition to the activities of the basic set of 13 carbohydrate enzymes also the activities and transcript levels of four different fructan activities enzymes have been determined. The level of water soluble carbohydrates including low degree of polymerization fructans were also measured. The results revealed distinct, cultivar dependent differences. Plant production faces tremendous challenges and climate changes already impacts agriculture significantly and requires robust and drought tolerant crops. Based on the hypothesis that deeper roots will allow plants to cope better with water stress and also take up nutrients more efficiently the RadiMax deep rooting screening facility has been established. It consists of four V-shaped pits of 400 m² with a sophisticated watering-system in which plants will receive irrigation only from the bottom, which ends at 3 meters depth, and moveable rainout shelters. 150 fixed-installed rhizotrons for semi-automated multispectral-camera systems allow studies of up to 150 different lines. Distinct above ground differences along the drought gradient for a specific *Lolium perenne* variety and also between different *Lolium* varieties could be detected by comparative above ground RGB-, thermo- and multireflectance imaging using drones, thus serving as proxy for differences in the root system.

Breeding perennial species to enhance sustainability of grassland based agriculture

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Productive grassland accounts for 65% of the UK's total agricultural area, with grass-based livestock production accounting for >60% of value. Grown together as a mixed sward, perennial ryegrass and white clover provide a high quality forage with white clover fixing up to 250kg N/ha/year that can be used by the grass companion. Because production of quality forage is key to grassland systems, nutrient application, including nitrogen (N) and phosphorus (P), is key to the agronomic performance of grassland agriculture.

Improving the efficiency of use of N and P in grassland systems through targeted plant breeding is a major challenge but also presents an opportunity for plant breeders to make a significant positive impact on the livestock sector by reducing production costs and environmental impact. Application of conventional and marker assisted selection for DM yield, persistence, forage quality and environmental traits have been highly successful with Aberystwyth varieties supplying approximately 70% of the UK seed market (Abberton et al., 2008). Similar approaches are now being used to target improved nutrient use efficiency (Marshall et al., 2012).

Genetic resources (populations and breeding lines of ryegrass and clover) have been being studied for their uptake and utilisation of N and P using a flowing solution culture system to identify germplasm for inclusion within the IBERS breeding programmes and to develop genetic markers that can be applied to the effective selection of these traits. Current research uses various genetically defined breeding populations and the National Plant Phenotyping Centre (NPPC) at IBERS to investigate N and P responses of this germplasm. The NPPC are developing non-destructive approaches to analyse plant growth and development, using non-destructive imaging technologies combined with traditional field based phenotyping. The presentation will present the current status regarding application of new phenotyping technologies to conventional and novel traits.

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Novel 3D Imaging System for Strawberry Phenotyping

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Accurate and quantitative phenotypic data is vital in breeding to assess performance of genotypes and make selections. Traditional strawberry phenotyping relies on the human eye to assess most external fruit quality attributes, which is time-consuming and subjective. 3D imaging is a promising high-throughput technique that allows multiple external fruit quality attributes to be measured simultaneously.

A low cost multi-view stereo (MVS) imaging system was developed, which captured data from 360 around a target strawberry fruit. A 3D point cloud of the sample was derived and analysed with custom developed software to estimate berry height, length, width, volume, calyx size, colour and achene number. Analysis of these traits in 100 fruit showed good concordance with manual assessment methods.

Our study demonstrates the feasibility of an MVS based 3D imaging system for the rapid and quantitative phenotyping of seven agronomically important external strawberry traits. With further improvement, this method could be applied in strawberry breeding programmes as a cost effective phenotyping technique.

Heavy soil and treated waste water result in reduced hydraulics and reduced levels of plasma membrane aquaporin (PIP) mRNA in citrus trees

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The concept of available soil water and physical strength for root water uptake and growths hypothesizes that heavy clay are less effective than sandy loam soil. Moreover, the concept of sorption hypothesizes that heavy clay adsorbs all the contaminant of applied water resulting in a toxic environment for roots. Irrigation with low quality water, such as, treated waste water (TWW) in heavy clay soil creates therefore unfavourable environment which might greatly reduce plant performance. In this study, we examine the effects of TWW, heavy clay, and sandy loam soil on growth, and hydraulic conductivity in comparison to fresh water (FW) and further elaborate on their effect on root aquaporin (CvPIP) gene expression of citrus trees. Almost all investigated parameters, photosynthesis rate, leaf transpiration rate, leaf relative water content, leaf water potential, root water uptake and whole plant specific conductance were lower in TWW as compared to FW and in heavy clay as compared to sandy loam, with lowest values under both TWW and heavy clay. The mRNA levels of eight CvPIP genes showed variable trends upon exposure to TWW and heavy clay; while six of them responded to TWW by reduced mRNA levels, two were unaffected in both soil types. Three PIPs (PIP1:2, PIP2:1, PIP2:2) showed significant reductions in their transcripts levels under TWW and heavy clay, with lowest values under both conditions. Moreover, the mRNA levels of these genes were in a good correlation with root hydraulic conductance, pointing their importance for water balance under optimal conditions. Although salt provides major component of TWW, the expression levels of PIP genes suggest that other components also contribute to the negative effect of TWW.

Dynamics of canopy and soil temperature variation in a Mediterranean vineyard

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Climate change imposes increasing restrictions and risks to Mediterranean viticulture. Heat and drought stress are becoming more frequent and severe. In addition, row crops such as grapevine, are more vulnerable to heat stress due to the amplified effect of soil surface on vineyard's heat fluxes which can be negative for grapevine physiology. Therefore, a better understanding of the dynamics of temperature patterns in soil and canopy can help to achieve a more precise vineyard monitoring and management. Ground based thermography was used to assess the dynamics of variation of canopy and soil temperatures along the day as means to predict plant water status, and evaluate potential risks of heat stress damage for leaves and berries. Field trials were carried along 3 consecutive years (2013, 2014 and 2015) in Alentejo (South Portugal) with two red *V. vinifera* varieties Aragonez (syn. Tempranillo) and Touriga Nacional, cultivated under deficit irrigation conditions. Leaf water potential and leaf gas exchange complemented thermal imaging. Canopy temperature (T_c) values were above the optimal temperature for leaf photosynthesis during large part of the day light period (11:00-14:00h to 17:00h), especially under high VPD and high T_{air} conditions. Soil temperature (T_s) was on average about 10-15°C higher than T_c . Our results suggest that T_s patterns can have an impact on T_c especially at the cluster zone. In addition, we found a strong correlation between T_c retrieved between 14 and 17h and leaf water potential and leaf gas exchange which re-enforces the relevance of T_c as an indicator. Our results show as well that T_s can be a feasible thermal indicator to monitor heat and water fluxes in Mediterranean vineyards.

Key words: Mediterranean viticulture, water and heat stress, thermal imaging, heat fluxes, soil and canopy temperature

Poster presentations:

Leaf photosynthetic characteristics in a willow (*Salix* spp.) and poplar (*Populus* spp.) pot trial in response to wastewater sludge treatments

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Some basic photosynthetic characteristics of young willow and poplar plants were studied in the course of a pilot pot trial as response to waste water sludge treatments. The experiment was designed in the context of an attempt for general assessment of bioproductivity in some fast-growing woody species. Standard cuttings (18-20 cm) of two hybrid poplar (*Populus* spp.) clones (I-214 and I-45/51) and white willow (*S. alba* L.) were used to establish a pot (ø 25 cm, 7.5 l) experiment with four different doses of wastewater sludge (1/5, 1/4, 1/3, 1/2 from the vessel volume plus control, WWTP, Kubratovo). Some photosynthetic parameters (net CO₂ assimilation, transpiration, and stomatal conductance) were measured during the first vegetation season. No significant positive effect of the wastewater sludge treatment on the net photosynthetic activity was detected in the poplar plants, but the clone I-214 showed higher values in all of the tested doses. While in clone I-45/51 a trend of increasing both the stomatal conductance and transpiration was recognized with raising the treatment dose, in clone I-214 the higher values of the same parameters were registered at the lower doses. The wastewater sludge supplement positively affected all the studied photosynthetic parameters in the willow plants indicating a clear trend of increasing the values with raising the treatment dose.

Breeding vine cacti with improved tolerance to heat stress

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The increasing threat of climate change is already having a substantial impact on agricultural practice worldwide in view of the impending risks for future global food security. A perennial fruit crop showing potential for the future for cultivation under rising temperatures would therefore be vine cacti of the genus *Hylocereus*, since the Crassulacean Acid Metabolism (CAM) of these species endow them with excellent tolerance to drought. Over the past few years, our traditional breeding program has focused on the production of superior hybrids in terms of fruit quality and few of them are currently being grown on a commercial scale. Three species were used for this purpose: the two diploid species *H. monacanthus*, with purple flesh and a red peel, and *H. undatus*, with white flesh and a red peel, and the tetraploid species *H. megalanthus*, with white flesh and yellow peel. Since tolerance to heat differs among *Hylocereus* species, expanded cultivation in drylands can be achieved only through hybridization and selection of elite cultivars tolerant to heat. We studied two of the hybrids produced in our breeding program (i.e., the allotetraploid Z-10 and the allotriploid S-75) and their parent species under short- and long-term heat stress. Short-term heat stress was studied by collecting leaf discs from mature stems and placing them in a water bath for 30 min at 35, 45, 55 or 65°C. Long-term heat stress was investigated by exposing rooted cuttings placed in pots to day/night temperatures of 45/35°C at 90% relative humidity for 14 days. Control plants were exposed to day/night temperatures of 26/20°C. Electrolyte leakage levels, availability of carbohydrates (soluble sugars in the form of glucose) and photosynthetic activity were determined. Overall, the results suggested that high temperatures could have significant effects on the physiological status of vine cactus plants and that the allopolyploid lines may be more tolerant to heat stress than their parental lines.

Phenotyping and genotyping of a mutant collection of giant reed (*Arundo donax* L.)

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Arundo donax L. (giant reed) is a perennial C3 Poaceae species of Mediterranean-subtropical origin and is characterized by high carbon accumulation efficiency, making it one of the most promising plants for bioenergy and phytoremediation exploitation. *A. donax* is highly polyploid, completely lacks sexual reproduction and propagates vegetatively. Very little genetic variation is found between ecotypes, which makes *A. donax* domestication and genetic improvement a challenge. We utilized physical mutagenesis with Gamma and Fast-Neutron irradiation treatments of *A. donax* in-vitro cultures in order to increase the genetic variability of the species. For the Gamma-ray treatments, LD50 was reached with irradiation doses between 40 and 60 Gy. Plants were regenerated, hardened in pots and transferred to field. A total of approximately 1,100 independently regenerated plants are currently available and mutants for plant height and habitus, leaf shape and color and other traits have been identified (Valli et al. 2017, GCB Bioenergy, 9, 1380–1389). We have additionally started to phenotype the collection for mutants affected in cell wall cellulose or lignin content, in order to identify clones potentially improved in bioenergy production. To our knowledge, these are the first results of artificially induced mutagenesis in *A. donax*. The molecular nature of induced mutations is being investigated with NGS-based genotyping means.

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Session 4: Phenotyping/Breeding for biotic stress tolerance

Oral presentations:

Quantification of biotic stresses on aerial parts of plants using Chlorophyll Fluorescence Imaging and Image Analysis

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Image analysis is increasingly used in plant phenotyping. Among the various imaging techniques available for plant phenotyping, Chlorophyll Fluorescence Imaging is particularly suitable for the imaging of biotic and abiotic stresses on the aerial parts of plants. Numerous chlorophyll fluorescence parameters may be measured or calculated, but only some of them may provide useful contrasts for the quantification of a given stress on leaves. In the perspective of high throughput phenotyping of biotic stresses on plants, we developed automated procedures to identify Chlorophyll Fluorescence parameters of interest for the quantification of a given biotic stress on large image datasets. The outputs of the automated procedures enable:

- the visualization of the whole dataset, by providing contact sheets for each of the chlorophyll fluorescence parameter tested.
- the visualization of basic statistics : radial-plot, box-plot and Mann-Whitney tests based on the mean intensity of each parameters are provided to compare the various treatments performed.
- images are clustered based on histograms associated to each images, thereby enabling the clustering of images leaves displaying leaves of similar phytosanitary status.
- the objective quantification of disease incidence on each leaf tested. The quantification can discriminate various stages of symptom development such as necrotic tissues, wilted and chlorotic tissues, and impacted tissues that do not display any symptoms visible to the eye.

Resistance to rusts: can we predict durability by complementation of field and growth chamber studies with histology?

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Rusts are major biotic constraints of many crops worldwide. Rust epidemics were so important already by Clasic Roman's times that they were symbolised in religious ceremonies like the Robigalia in honour of the rust god Robigo. The scientific basis of disease resistance breeding did not start till one hundred years ago after demonstration that resistance is inherited in a Mendelian way. Soon, extensive breeding programmes were initiated in many countries, and numerous rust resistant cultivars have been produced by using single genes, many of them introduced from wild species. Unfortunately this highly specific, clearly recognised complete resistance is often ephemeral due to the evolution of virulent fungal isolates that negated the breeders efforts and lead to spectacular "boom and bust" cycles.

This has reaised an increasing concern on durability and in the search of more durable types of resistance. There has been some degree of success in achieving durable resistance even though we still do not fully understand what actually causes a resistance to be durable, nor how to distinguish durable resistance from non-durable resistance, nor even what criteria to use to decide whether a resistance is durable. We present some examples where resistance to rusts that proved to be durable is of complex inheritance, and also others where durability is due to particular single genes that cause relatively small effects on the pathogen development, and yet seem to be race-non-specific and not based on hypersensitivity. Combinations of different resistance mechanisms seem to be of great value to increase durability of resistance. Some examples will be provided and critically discussed.

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Phenotyping *Castanea* hybrids from controlled crosses for resistance to *Phytophthora cinnamomi*

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Castanea sativa is susceptible to *Phytophthora* spp., a serious root pathogen causing root rot, while *C. crenata* and *C. mollissima* show resistance to infection. Inter-specific controlled crosses were established between *C. sativa* x *C. crenata* and *C. sativa* x *C. mollissima* for introgression of resistance genes from the resistant species into the susceptible *C. sativa* and two mapping populations were produced. These crosses had two purposes: the first to create mapping populations to identify Quantitative Trait Loci (QTL), genetic factors that control ink disease resistance, the main threat that affects *Castanea sativa* still nowadays in Europe, the second, to select resistant hybrid genotypes for rootstocks for propagation and delivery to the industry which presents a serious deficit of improved plant material in Portugal and Europe. *Phytophthora cinnamomi* susceptibility of each progeny was evaluated by root and excised shoot inoculation tests. For each test multiple copies of each genotype were used. The number of days of survival after root inoculation was the best discriminator of resistance to *P. cinnamomi*, while the percentage of shoot with internal lesion was the most explanatory symptom associated with survival. The lesion progression rate in the excised shoot inoculation test, was strongly and positively correlated with the survival in the root inoculation test. The excised shoot inoculation test appears to be a reliable and expedite method for screening large numbers of genotypes regarding resistance to *P. cinnamomi*, which is very useful for mapping purposes. An overview of the entire breeding program in progress will also be presented, with the main results obtained so far (1,2,3,4).

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Phenotyping *Pisum sativum* germplasm for resistance to aphid (*Acyrtosiphon pisum*) and weevil (*Bruchus pisorum*) under field and controlled conditions

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Pea weevil (*Bruchus pisorum*, *Bp*) and pea aphid (*Acyrtosiphon pisum*, *Ac*) are highly destructive pests affecting pea (*Pisum sativum*) production worldwide. *Bp* larvae feed into seeds decreasing the quality and marketability of the seeds. *Ac* is a sap-sucking insect of several legumes species, which causes significant yield losses and transmit viruses. Chemical control is possible but requires repeated treatments with the associated detrimental environment effects.

Here we report the phenotyping studies performed in our department to identify and characterize pea resistance against both pests. Field assessment consisted on monitoring plant traits that might contribute to scape mechanisms, and assessing pest incidence and severity and plant damage. For *Bp* separate assessments were made on percentage of infected seeds, and the level of larval development as they can reflect possible antixenosis and antibiosis mechanisms, respectively.

In addition to field observations, we performed no-choice and multiple choice bioassays with selected accessions under semi controlled and controlled conditions to corroborate and characterize the resistance mechanism detected in field. This allowed the elucidation of antixenosis, antibiosis and tolerance mechanisms.

ACKNOWLEDGEMENTS: This work was funded by Spanish AGL2014-52871-R and EU-FP7-LEGATO projects

Presymptomatic leaf disease detection with a five-band multispectral imager

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Plant abiotic and biotic stresses impact yield and fruit quality. Hence, a premature identification of the stress helps to avoid quality and crop losses. Microorganisms such as bacteria and fungi often cause severe diseases, which in turn could be controlled by deploying pesticides. *Botrytis cinerea* is one of the most damaging necrotrophic fungi attacking hundreds of different plant species. In tomato, *Solanum lycopersicum*, it attacks leaves, stems and fruits. A presymptomatic identification could allow an efficient fungicide regime by applications only when the disease is present.

In artificial inoculation assays under controlled environmental conditions, we infected detached tomato compound leaves with *B. cinerea* and ran time-lapse experiments using a multispectral imager (RedEdge, Micasense, Seattle, WA, USA). This imager collects data in five 10-40 nm wide bands (red, blue, green, RedEdge and NIR (near infrared)) through individual lenses on one chip. After applying dark frame subtraction, lens de-vignetting, lens undistortion and intensity-based band alignment, an alignment error of two pixels remains due to parallax effects. A supervised RedEdge-based classification approach allowed for the presymptomatic identification of infected leaf tissue within 24 hours post inoculation (hpi). In fact, after manually tweaking classification thresholds we were able to identify diseased spots even earlier at approximately 16 hpi. Shapes of infected but presymptomatic tissue obtained with multispectral, low-resolution camera were then compared to RGB imagery of visible symptoms taken three days post inoculation. The results suggest a detection of roughly 75% of symptomatic leaf area by multispectral imaging in a presymptomatic phase (24 hpi). This approach is adapted to controlled environmental conditions. Future work will look at the challenges under day light conditions.

Polyploidy influences *Malus x domestica* / *Venturia inaequalis* interactions

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In this study, performed at KU Leuven in years 2016-17, we aimed at unravelling the effect of polyploidy and defense priming with fosetyl-aluminium in apple (*Malus x domestica* Borkh.) on resistance to apple scab (*Venturia inaequalis*). At first macroscopic disease symptoms were analyzed in the apple scab susceptible (primed and unprimed) diploid and tetraploid genotypes. Next, the evolution of *V. inaequalis* DNA at the molecular level was quantified via qPCR analysis. Finally, we performed RNA-sequencing to analyze differences in gene expression upon the various treatments.

Macroscopic scab symptoms in the leaves indicated different degrees of susceptibility with the highest degree of symptoms in unprimed diploid plants, and the lowest in primed tetraploid plants. Over all the treatments, we could conclude that polyploidy in Gala reduced sporulation by 38,3 % in comparison to the diploid genotype. Results were confirmed with qPCR. The amount of *V. inaequalis* DNA was decreased by 63,5 % in unprimed and 61,5 % in primed tetraploid plants in comparison to diploids. Moreover, significant differences between diploids and tetraploids were identified in all transcriptomic comparisons.

We can conclude that polyploidy substantially improves the resistance of apple plants to *V. inaequalis*. The effect can even be increased by priming the defense response. The observed beneficial phenomenon is supported by differences in gene expression profiles.

Poster presentations:

Managing on-farm biosecurity risk through pre-emptive breeding: rust of peas and lentils

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Rust is a significant yield constraint in the production of lentil and field pea in various regions of the world. Rust is incited by *Uromyces viciae-fabae* in lentils and in peas under tropical conditions, while *U. pisi* is the principal agent causing pea rust in temperate regions.

A pea and lentil germplasm collection consisting on more than 300 accessions including current elite cultivars and breeding lines selected in key rust host-spots, were evaluated in terms of rust resistance under field and controlled conditions to allow the identification of novel sources of resistance and introgression of resistant loci into locally adapted elite germplasm. Plants were artificially inoculated and were macroscopically evaluated under field conditions at Córdoba, Spain during two growing seasons. In addition, germplasm was also evaluated under controlled conditions against 4 different rust isolates from various origins. Plant responses were assessed in terms of infection type (IT) and percentage of disease severity (DS %). The most common response of pea to *U. pisi* was of susceptibility, with all pea accessions showing compatible reaction with mature pustules (IT=4) although 17% of them showed partial resistance associated with reduced DS (<10%). Similarly, susceptibility was also very frequent in lentil accessions against *U. viciae-fabae*, although in this case low IT was identified. Still, 15% of the accessions showed DS < 10%. These results were validated under controlled conditions with isolates from diverse geographical origins.

Selected resistant accessions are being crossed with elite Australian germplasm and markers associated with rust resistance will be developed.

ACKNOWLEDGEMENTS: This work was funded by Australian GRDC and supported by the CCDM - a joint initiative of Curtin University and the GRDC.

Preliminary results of use of thermal imaging in sunflower phenomics

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High-throughput and highly accurate phenotyping methods are needed for biotic traits, where rapid, precise, and reproducible quantification of symptoms is required for estimating the disease severity and evaluation of host resistance. Up until now, qualitative visual assessments have often been used to quantify disease severity. They require rating scales to be finely tuned, and raters to be trained, which increases considerably the cost and the time devoted to phenotyping projects. In contrast, automated image analysis-based phenotyping provides a powerful alternative to qualitative visual assessments. In particular, recent advances in thermal imaging have made it feasible to assess plant-pathogen interactions by monitoring patterns of surface tissue temperature. Here we report the results of a preliminary study aimed at testing of the practical utility of thermal imaging for resistance screening of *Sclerotinia* head rot (SHR) and *Sclerotinia* stem rot (SSR). We used an early flowering sunflower genotype highly susceptible to *Sclerotinia sclerotiorum*, and acquired images 7 days post inoculation (dpi) for stem, and 30 dpi for the capitula. Temperature differences up to 4°C were observed between the healthy and diseased tissue. The contrast between unaffected and lesioned areas on thermal images was markedly higher than in visible images, potentially facilitating automated quantification. These results suggest that thermal imaging could be highly suitable for disease quantification as well as for remote sensing approaches.

Phenotypic changes in camelina plants caused by pathogenic fungus *Albugo candida*

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A field trial with 54 camelina genotypes was set up at production plots of Institute of Field and Vegetable Crops at Rimski Šančevi. It was sown on March 29, 2016. The trial was set up with an aim to test a possibility of breeding and growing camelina in agroecological conditions of Northern part of Serbia. Visual monitoring of genotype resistance (tolerance) to disease occurrence caused by pathogenic fungus *Albugo candida* was part of testing. The first disease symptoms on plants were spotted around May 10. Phenotypic changes on plant aboveground parts were manifested as deformations and shortening of internodes, twisting leaves and lagging in growth. In addition to stunted appearance of stem and leaves, thick creamy-white mycelia were observed on infected plants. There was no flowering and seed formation in such systemically infected plants. Humid weather in late May and early June favoured the spread of symptoms in plants. In this period (flowering stage), the occurrence of fungus mycelium was observed in individual branches, flowers and later on shells based on visual examination. Due to the presence of parasites, the phenotypic changes were expressed as deformation and hypertrophy of individually affected branches and floral bundles. The flowers were mostly sterile on such blossoms; in places where seeds were formed, it was mainly shrivelled. Besides monitoring the symptoms occurrence, resistance/sensitivity of tested genotypes was assessed. The first evaluation was done on May 22, and the second on June 07, 2016. Plants with present symptoms were considered sensitive, while those with no visible symptoms from emergence to ripening were considered resistant. Based on variance analysis ($P < 0.01$) genotypes marked 8, 34 and 50 have showed significant sensibility to pathogenic fungi *Albugo candida*, while other genotypes were not statistically significantly affected. Data obtained by phenotyping will be used in camelina breeding program for biotic stress tolerance.

Key words: *Albugo candida*, phenotypic changes, genotypes, camelina, symptoms

This work was funded by the Serbian Ministry of Education, Science and Technological Development (project TR31025) and Serbian-Austrian bilateral cooperation project 451-03-01039/2015-09/42.

Stripe rust severity assessments and its relations to winter wheat yield losses

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Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating wheat diseases worldwide. It became an economically important pathogen in Serbia in 2014 when it caused severe wheat yield losses. In order to quantify the severity of stripe rust in the field, a number of various disease assessments have been used. The objective of this study was to determine the relationship between stripe rust severity assessments and yield losses.

The field trials were conducted in the locality of Rimski Šančevi in Vojvodina, the northern province of Serbia, in 2014 under naturally occurring inoculum. Forty wheat varieties used in this study were sown in the plot sized $10 \times 5 \text{ m}^2$. Stripe rust severity was assessed in early milk stage of growth according to the modified Cobb scale (Peterson et al., 1948). This scale, based on the percentage of leaf area affected, was applied on the flag (F), penultimate (F-1) and antepenultimate (F-2) leaves of the main stems of ten randomly selected plants in each plot. Grain yield (kg/ha) of each plot was measured at 15% water content. Yield losses (%) were calculated based on the highest grain yield response of the tested varieties obtained from the Vojvodina locality. Linear regression model and Pearson coefficient of correlation were applied to determine the relationship between stripe rust severity on F, F-1, F-2, sum of the affected leaf area on F, F-1, F-2 and grain yield and yield losses.

Stripe rust was the only disease detected on the tested varieties. Disease severity ranged from 0% to 100%, and yield losses ranged from 14.2% to 84.0%. High positive correlation was found between all assessments of stripe rust severity ($P=0.000$). In order to find out which type of stripe rust severity assessment had greater correlation with estimates of yield losses, the data were analysed in two ranges of disease severity. When disease severity was up to 30% high positive significant correlation ($r=0.792$, $P=0.01$) was detected between sum of the affected leaf area on F, F-1, F-2 and yield losses. There were no correlations between other types of assessments and yield losses. However, if data included assessments of a broader range of disease severity (up to 100%) there was significant positive correlation between all types of disease assessments and yield losses ($P<0.05$). These results indicated that in years with lower stripe rust disease incidence it is important to sum up the assessments from all affected leaf area in order to estimate the impact of stripe rust on grain yield and yield losses. However, it is also important to point out that after regression analyses, relationship between stripe rust severity and yield loss was shown not to be always straightforward and other abiotic and biotic factors affecting yield formation should not be neglected.

Sunflower breeding for biotic stress resistance

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The main objective of NS sunflower (*Helianthus annuus* L.) breeding program is development of high-yielding hybrids with high oil content, stable and tolerant to biotic and abiotic stresses. Sunflower diseases represent a limiting factor in production and can cause severe losses of yield and quality. There are over 30 different pathogens which infest sunflower. A major problem in breeding for resistance is the existence of several races of certain pathogens. Breeding to promote resistance to these diseases is considered the most effective way of controlling them. Sources of resistance or different levels of tolerance to most diseases should be sought through wild species of the genus *Helianthus*. Certain wild species have rendered genes of resistance to downy mildew (*Plasmopara halstedii* Berl. & De Toni), rust (*Puccinia helianthi* Schw.), wilt (*Verticillium* sp.). High level of tolerance to *Phomopsis/Diaporthe helianthi* Munt.-Cvet. and *Macropomina phaseolina* has been obtained, and a certain level of tolerance to white rot (*Sclerotinia sclerotiorum* Lib. de Bary) and phoma black stem (*Phoma macdonaldii* Boerama). There is still a large number of diseases for which resistance sources remain to be found. Another limiting factor in sunflower production is a parasitic plant, broomrape (*Orobanche cumana* Wallr.). Broomrape is present in all sunflower growing regions except at the American continent and can drastically decrease seed yield. A major obstacle in breeding for broomrape resistance is constant emergence of new races, therefore finding the new sources of resistance is necessary. Wild sunflower species have successfully served in finding genetic sources (genes) of resistance to broomrape and its transfer to the culture form. The final result of this intensive breeding program is over 500 released hybrids in Serbia and other countries.

Rapid high-throughput phenotyping of an EMS mutant platform of *Cucurbita pepo* for tolerance to abiotic and biotic stresses

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To improve our understanding of gene function, while identifying interesting agronomic traits for breeding, an EMS mutant platform was generated in zucchini squash, consisting of 3,751 independent M2 lines. The collection was phenotyped for important agronomic traits such as ethylene insensitivity, and tolerance to biotic and abiotic stresses. By using the triple response of etiolated seedlings to ethylene we have screened the complete library (about 37,000 plants), which resulted in four ethylene-insensitive mutants. Adult mutant plants were then phenotyped, searching for alterations in vegetative development, sex expression, flower and fruit development, and postharvest fruit quality, thus exploring the potential uses of these mutations in squash breeding.

Given that abiotic stress tolerance is commonly associated with tolerance to oxidative stress, a rapid phenotyping method was developed for the screening of 1,700 mutant lines for H₂O₂ tolerance. For this purpose 10 seeds of each line were embedded and germinated in 1% H₂O₂ and 0.05 mM of the catalase inhibitor aminotriazole, and the radicle length assessed using digital image processing. Several mutants were detected showing higher and lower tolerance to oxidative stress. Seedlings and adult plants of these mutants are currently being evaluated for tolerance to water and temperature stresses.

Regarding resistance to biotic stresses, the collection was screened for tolerance to *Tomato leaf curl New Delhi virus* (ToLCNDV), a begomovirus causing severe damages in Zucchini and other cucurbit crops. After phenotyping more than 1,000 lines we identified a putative recessive mutation conferring ToLCNDV resistance. Although traditional phenotyping approaches are proving successful, the massive screening and the use of mutant collections will require the development of more effective and faster non-destructive phenotyping methods, as well as their integration into innovative plant breeding programs.

Biochar induced bacterial and fungal microbiome promotes plant growth and mediates systemic resistance in tomato against soilborne disease

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Biochar, in addition to carbon sequestration, soil amelioration and improvement of plant performance, can significantly reduce plant diseases. Nevertheless, the mechanisms associated with soilborne-disease suppression and plant growth promotion are not fully understood. This study tested the effects of biochars on fusarium crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. *radices-lycopersici* (FORL), with an emphasis on mechanisms of disease suppression and plant growth promotion. Biochar amendment enhanced plant performance and suppressed FCRR of tomato. Culturable counts of beneficial bacteria and fungus was significantly increased by biochar and displayed antagonistic activity towards FORL. Illumina sequencing analyses of 16S rRNA and ITS gene showed substantial differences in rhizosphere and rhizoplane bacterial and fungal taxonomical composition between biochar-amended and control soils. Nevertheless, biochar amendment caused a significant increase in microbial taxon and functional diversity, microbial activities and an overall shift in carbon-source utilization by microbial communities, concurrent with increased plant growth and disease suppression. In order to determine whether systemic induced resistance (IR) might be involved in disease suppression, the site of biochar treatment and the site of pathogen inoculation was spatially separated by crown/stem inoculation approach. Furthermore, tomato mutants from various genetic backgrounds modified in the phytohormones salicylic acid (SA), jasmonic acid (JA), or ethylene (ET) metabolism were used to identify pathways involved in IR. We found that biochar-mediated systemic induced resistance in tomato against FORL and requires JA and ET pathway but not SA. JA and ET pathways are commonly triggered by beneficial rhizosphere microorganisms (PGPR and PGPF). This study indicates that biochar-elicited beneficial microorganisms is playing an essential role in biochar-mediated IR against soilborne diseases and enhanced plant growth promotion in tomato.

Phenotyping under realistic abiotic and biotic stress conditions the HMGU –SCREENs

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In the frame of EPPN2020, the Institute of Biochemical Plant Pathology (BIOP) and its associated Research Unit Environmental Simulation (EUS) at the Helmholtz Zentrum München provide Transnational Access (TA) to three plant phenotyping platforms. The sophisticated environmental simulation platforms enable experiments under realistic and reproducible multi-stress conditions (gas composition, above- and below-ground climate, air pollutants, pathogens/microbes, etc.) either in walk-in exposure chambers (ExpoSCREEN) or in solar simulators (SunSCREEN). Globally unique, the phytotron facility allows simulation of natural sun light for the mid latitudes, in particular of the short wave UV radiation (UV-B: 280-315 nm).

The capabilities of Expo- and SunSCREEN are splendidly suitable for long-term multifactorial experiments relevant for phenotyping and for understanding plant adaptation, plant-atmosphere and plant-microbe interactions under historic, present and future environmental conditions.

A novel HTP confocal microscopy platform (SignalSCREEN) enables analysis of roots and shoots for disease susceptibility and pathogen defence phenotypes. SignalSCREEN is dedicated to phenotyping early events in plant-microbe interactions on the seedling and young plant level including the application of fluorescently labeled

Session 5: Phenotyping/Breeding for abiotic stress tolerance

Oral presentations:

Phenotyping for drought tolerance in wheat using complex stress diagnostic system

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Drought is one of the most important abiotic stress factors in Carpathian basin and depending on the season, it can seriously limit wheat production at South Hungarian Great Plain. Breeding for drought tolerance is becoming more and more important challenge in crop plants, notably in wheat. The breeding process includes the characterization of the basic breeding materials in aspect of performance under well watered and drought stressed conditions. For experimental and breeding goals, we set up a complex stress diagnostic system in the greenhouse of the Cereal Research Non-profit Limited Company where we could analyze the responses of different wheat genotypes to drought. The control wheat plants were grown under ideal water regime (watering to 60% of the 100% soil water capacity) and under drought stress conditions (watering to 20% of the 100% soil water capacity). The effect of water withholding on plant growing was tracked by a digital imaging system based on number of plant pixels. After harvesting, plant heights, spike lengths, grain numbers and total grain weights were measured and values of well-watered and stressed plants were compared. Among the pre-selected wheat genotypes/varieties significant differences were registered. The methodological details and the results were demonstrated in the lecture and practical demonstration.

A genome-wide approach combining field and platform phenotyping to investigate plant responses to drought and high temperature

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Natural genetic resources need to be fully explored for designing novel genotypes able to cope with climate changes, with special emphasis on identification of traits and allelic sources of adaptation to drought and high temperature. We base our approach on a combination of phenotyping methods allowing genetic analyses in phenotyping platforms and in the field. The rationale is that a given trait or allele confers advantages for yield in specific scenarios of water deficit or high temperature, but most often not in all of these scenarios. We have explored, in a network of field experiments, a series of environmental scenarios for identifying in which scenarios a given allele has positive, negative or no effect on yield. We have then dissected these effects into responses to specific environmental conditions and their genetic variability. The latter step involves phenotyping in an automated phenotyping platform, allowing one to infer traits that are impossible to measure in the field, such as radiation use efficiency, sensitivity of growth to water deficit or stomatal control. We have applied this strategy to a panel of 250 maize hybrids allowing a multi-scale multi environment whole-genome association study. Resulting pattern of QTL effects expressed as function of environmental variables and traits can be used for assessing the performance of genotypes and the contribution of genomic regions under current and future stress situations, and for accelerating the breeding for drought-prone environments. Hence, we believe that there is no point in opposing phenotyping in the field and in controlled conditions. Our experience shows that the combination of both approaches allows gaining insights in the genetic control of yield in diverse climatic scenarios.

Phenotyping preselected somatic hybrids of potato with the wild species *Solanum chacoense* and *S. bulbocastanum* for drought tolerance

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Somatic hybrids between potato cultivars (tetraploids) and the wild diploid species *Solanum chacoense* (*chc*) and *S. bulbocastanum* (*blb*) were produced previously through protoplast electrofusion. In the case of *chc* somatic hybrids two different strategies were used: wild type *chc* (an accession with the highest leptine content = HL) and *chc* HL transgenic for the gene *MSH2*, involved in the mismatch repair system (MMR). The transgenic plants carrying *AtMSH2* gene in antisense orientation or a mutated form (dominant negative gene), were proven to be deficient in the MMR system. They also were prone to mutations and recombination at somatic level by previous analysis of resistance to Colorado potato beetle.

For both combinations somatic hybrids and back-cross progenies were used and preselected *in vitro* for tolerance to drought by using two concentrations of PEG 6000 (5% and 15%). After *in vitro* selection the most promising clones, parental lines and some negative controls sensitive to drought were multiplied and transferred to HAS-RSDS Szeged EPPN phenotyping platform. The plants were acclimatized, and three replicates were used to evaluate drought stress, comparing controls (60% water soil capacity) to the drought stress clones (20% water in soil). The experiments were carried for six weeks and based on image analysis biomass accumulation was recorded as well as photosynthesis at two time points by using fluorometers. The results revealed that for somatic hybrids between potato cv. Delikat and *chc*, with or without MMR deficiency, although the parents are sensitive to drought some of the somatic hybrids with MMR deficiency were able to recover and produce a biomass not significantly different from controls. The somatic hybrids with *blb*, were also able to overcome the medium drought stress as the wild parent. Some of the BCs were also showing drought tolerance based on biomass accumulation. Tuber yield varied in greenhouse and some of the clones did produce the same or even more tuber yield than controls. Photosynthesis was not affected in any combination, proving that potato can overcome drought by maintaining its photosynthetic activity. The correlations between drought tolerance and resistances to biotic factors in relation with genetic constitution of the somatic hybrids are also going to be discussed. Somatic hybrids phenotyping in potato brings new valuable data for their use in breeding programs.

High throughput growtainer phenotyping combined with on-line transpiration monitoring to select water efficient cultivars: a proof of principle in the banana bio(di)versity collection

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An important crop that needs vast amounts of water for optimal production is banana (*Musa* spp.). The International Transit Center of Bioversity International holds the world's biggest collection of banana biodiversity (>1500 accessions).

Our aim is to evaluate the potential within this collection by phenotyping for climate smart agricultural usage. We have characterized the growth potential of 32 representatives of the *Musa* biodiversity and have ranked their growth under control and mild osmotic stress conditions. 5 cultivars, representing 51 related accessions in the collection, were found to have superior growth. This growth models are being scaled up to a growtainer. Our growtainer simultaneously grows 504 plants.

To get more insight into the physiology, we monitor in a next step the individual plant transpiration of selected genotypes gravimetrically. We gained already insight in the day to day, and within day, variations of transpiration rate, of all 32 representatives under controlled conditions. We show that banana declines its transpiration rate near the end of the day, reducing transpiration water loss up to 30%. This feature is correlated to efficient water consumption and is thus a valuable, rapid screening parameter for drought tolerance. We hypothesize that this might be a feedback from photosynthesis. With sufficient root pressure and less need to fix CO₂, increase the stomatal resistance for gas exchange results in a higher water use efficiency. We have selected 25 phenotypic variables related to the transpiration pattern. Based on blind clustering within the 25 dimensional space we detect two major feedback phenotypes in the *Musa* biodiversity: water consuming cultivars with a late feedback, and cultivars with a balanced water usage. Breaking up, and describing the daily transpiration patterns proves useful as a cultivar selection tool, while also allowing insight in the plant physiology of transpiration.

Synergistic effects of salt and drought stress in wheat responses studied by high throughput phenotyping

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Screening of crops for tolerance can strengthen breeding programs by identifying genotypes with increased tolerance and yield potential. The main objective of the present study was to analyze salt tolerance of 14 wheat genotypes originating from different geographical locations (Austria, Azerbaijan and Serbia) under well-watered and water limited conditions by using high-throughput shoot and root phenotyping platforms. Comparative investigations of various morphological, physiological, biochemical and enzymatic responses concerned with salt and drought tolerance were performed at different developmental stages. Our results show that the adverse effects of salt stress are enhanced under water limitation. Water use efficiency remains unaffected by salt stress in well performing wheat lines, e.g. Capo, but drops significantly in salt sensitive lines, e.g. Azamatili 95. Analysis of gas exchange parameters during biomass accumulation and grain filling period show that biomass accumulation and grain production is roughly correlated with photosynthetic efficiency. Proline is an important osmoprotectant whose amount was significantly increased under salt plus drought stress, and also to a smaller extent under drought stress alone, while the applied salt stress alone had negligible effect on the proline content. It is concluded that the co-occurrence of saline and water limited conditions induce large extent of biomass and grain yield loss in wheat, which can be partly reversed by providing well-watered conditions if it is practically feasible. We could also identify wheat lines which show higher tolerance to combined effects of salt and drought, which demonstrates the potential of high throughput plant phenotyping in breeding for drought and salinity stress tolerance in crop plants.

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Root growth and anatomy of spring wheat in response to waterlogging

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An increase in heavy precipitation events and a higher risk of floods are likely consequences of projected climate changes in many parts of the world. Improving the waterlogging tolerance of wheat varieties could alleviate yield constraints caused by excessive rain and poor soil drainage. Six spring wheat genotypes with contrasting waterlogging tolerance properties were selected for a greenhouse study investigating root growth and anatomical root traits in response to waterlogging. The experiment was conducted in a randomized split-plot design using rhizotrons. A waterlogging treatment was imposed at the three leaf stage and lasted for seven days. Images of roots and shoots were acquired every second day investigating the stress response. Measurements were taken prior to, during and after the treatment. At harvest, root segments (2 cm) from the longest seminal and nodal root of each plant were sampled at three zones (upper half, the middle of the root, lower half). Images of root cross sections were acquired with a light microscope and analyzed in ImageJ for the following anatomical root traits: cross sectional area, cortex area, stele area and percentage of aerenchyma. Results show that waterlogging tolerant genotypes developed a higher number of seminal and nodal roots, which resulted in a greater total length of each root class per plant under waterlogged conditions. In all genotypes, seminal root elongation ceased during the treatment but resumed root elongation after the treatment. During the recovery period, tolerant genotypes developed a mean seminal and nodal root length that was 5.6 and 9.6 cm larger than for sensitive genotypes. Tolerant genotypes had a higher percentage of aerenchyma in nodal roots exposed to waterlogging. In addition, tolerant genotypes had a significantly smaller stele in seminal and especially in the nodal roots. The diameter of the stele was smaller for plants in both treatments, indicating that it is an inherent trait rather than an adaptation. A smaller stele size likely reduces the oxygen demand under anaerobic conditions and might be particularly beneficial in combination with aerenchyma. Our results suggest that stele size is a trait relevant for investigations in larger populations.

Molecular mapping of root traits in durum wheat in environments with lower water availability

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Durum wheat (*Triticum turgidum* L. var. *durum*, $2n = 4x = 28$) is one of the most important crop for the agriculture and economy of Mediterranean countries. Understanding the genetic diversity and the genotype population structure based on molecular markers at the genome level can speed up the utilization of diverse genetic resources for varietal improvement. In the present study, the association mapping (AM) strategy based on a panel of 183 elite accessions from different durum wheat growing regions of Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico and also a population of 176 recombinant inbred lines (RILs) derived from the cross between two Italian elite durum wheat cvs. Meridiana and Claudio, were deployed in order to identify QTLs for root traits and compare their overlaps with other QTLs identified. The panel was profiled with simple sequence repeat, Diversity Arrays Technology and sequence-tagged site markers. The genetic relationships among the accessions have been investigated using both a genetic-similarity and a model-based Bayesian clustering method. Significant differences among genotypes were observed for all measured root traits. In this direction, our study enabled us to map two major QTLs controlling seminal root angle on chromosomes 4B and 6B. For these two QTLs, along with others, we provided molecular markers associated to the favorable allele. In general, at these QTLs, a positive association between root length and agronomic performance was observed, mainly in environments with lower water availability.

Functional genomics and phenomics: focusing on plant protection products' R&D

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The comprehensive correlation between plants' phenomes and metabolomes could facilitate the development of robust phenomics models for applications in pesticide R&D. Plant stresses by biotic or abiotic factors cause substantial metabolic changes that can be recorded applying metabolomics. Focusing on crop protection, plant protection products (PPPs) exert their bioactivity by interfering with biochemical systems or processes that are vital for the survival of the target-organisms. However, although PPPs have a primary mode-of-action (MoA), many of them have also secondary MoA, thus, they interfere with other systems of lesser importance for the target- or non-target organisms. Among PPPs, fungicides is an important group intended to protect crops from phytopathogenic fungi, affect systems such as, the electron transport, membranes, or various biosyntheses. However, plant and fungi share many biochemical systems making the selectivity of fungicides a challenging task and information on their effects on crops, is largely fragmented. Within this context, as a proof-of-concept, we have undertaken the task of studying the effect of selected fungicides on the metabolism of plants, using lettuce (*Lactuca sativa* L.) as the model plant, applying metabolomics. Results revealed a variable effect of the fungicides on the primary and secondary metabolism of the plant, with various pathways being up- or down-regulated. Also, metabolites-biomarkers of the effect of the fungicides on plant's metabolism were discovered, many of which such as, trehalose, L-proline, and GABA, play important role in their physiology. Results could be further integrated with results of phenomics in early stages of PPPs' R&D in order to gain insights into their effects on plant physiology helping towards their optimization and plausibly the selection of plant varieties based on their responses to the applied PPPs.

Association mapping for fiber traits and drought tolerance in elite cotton (*Gossypium hirsutum* L.) germplasm

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Cotton has an important position in the textile, pharmaceutical, oil and food industries in the world. The textile industry is largely based on cotton fiber. Cotton fiber is more preferable to other vegetable and synthetic fibers because of sweat-absorbing, less transmission of static electricity, air permeability of natural fibers. As a result of fiber quality, fiber yield directly affects economic value of cotton production. Drought stress which causes seriously yield losses up to 70-80% is an important problem for cotton production throughout the world. In view of the effects of drought and fiber quality in cotton production, the improvement of fiber traits and the development of drought tolerant individuals are crucial to agronomic industry. For this purpose, using conventional breeding methods are time consuming and laborious. Instead of conventional breeding methods, association mapping is favourable and fast alternative method. Determination of significant trait-associated markers allows their use in molecular breeding studies. In our study, we aim to develop cotton cultivars with drought stress resistance and high quality fiber features using association analysis. To achieve our aim we used 177 SSR markers providing 967 loci in a panel composed of 105 different cultivars (*Gossypium hirsutum* L.). The population was subjected to drought stress by growth under limited irrigation conditions. Under limited and control conditions, 22 agronomic features were measured. Population structure was determined with STRUCTURE 2.3.3. Phenotypic and genotypic data were associated using TASSEL 2.1 to find associated markers with agronomic traits. Different sets of SSR loci were associated with agronomic traits under irrigated and test conditions ($p < 0.01$). The identified markers can be used in marker assisted selection mediated breeding methods. The outputs of our study will be helpful for development of drought resistant cotton cultivars using molecular marker technologies.

Phenotyping for heat tolerance - from lab to field

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The aim was to correlate the heat responses of young tomato (*Solanum lycopersicum* L.) from controlled environments to field using the maximum photochemical efficiency of PSII (Fv/Fm) and gas exchange. Initially 28 tomato genotypes, selected for good performance in the field in Nepal were phenotyped in 40/28°C day/night temperature for two days in controlled environments followed by a second screening of four genotypes with high and four with low Fv/Fm for four days in 38/28°C, followed by 5-day recovery in 26/20°C control. The genotypes with high Fv/Fm were able to maintain high rates of net photosynthesis (PN) at 38°C, due to high stomatal conductance (gs) leading to efficient cooling of the leaf. The four genotypes had identical biomass during control but after heat stress a difference between the sensitive and tolerant groups were seen in biomass accumulation and pollen viability, similar to Fv/Fm and PN. Two tolerant and two sensitive genotypes were grown with irrigation in the field in Nepal during a natural heat wave (38/26 °C). The tolerant genotypes had significantly higher biomass accumulation; lower heat injury index and higher fruit yield than the sensitive genotypes.

To our knowledge this is the first study where screening of tomatoes for heat tolerance by Fv/Fm in controlled environments have been followed by a field trial for agricultural traits under natural high temperature stress. The results confirm Fv/Fm as an effective physiological marker for early detection of heat tolerance and correlates to the yield under stress in the field.

Whole-plant stress performance analysis: a new tool for functional phenotyping

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Abiotic stress factors are critical limiting factors for crop yields and long-term food security. Despite spectacular improvements in our “omics” tools, a large gap exists between those research investments and the commercial release of stress-tolerant crops. It is widely accepted that phenotyping tools are necessary to reduce this gap, in general, and understand the complexity of interactions between environment and genotype (G \times E), in particular. Here we describe a new high-throughput experimental physiological phenotyping platform that is based on a gravimetric system combined with a unique irrigation-drainage system, as well as soil and atmospheric probes. This system enables tight control of multiple soil water/salinity scenarios, as well as simultaneous and continuous monitoring of the soil-plant-atmosphere water relations across a population of plants. Using this functional system, we screened 81 different barley lines under control, water deprivation and recovery scenarios. In order to transform the phenotypic data into applicable knowledge, we developed a performance-analysis tool that is based on multi-factorial analysis of variance and scoring of all physiological traits. Each plant was profiled based on its particular physiological responses to drought and recovery. We then chose 18 lines with different behaviors and repeated the same experiment with at least five biological repetitions for each. A Venn diagram analysis between the lines with the highest scores for growth rate, water-use-efficiency and stomatal conductance identified two lines with improved efficient water-use (EWU). Moreover, we found that the quantitative physiological traits that we measured were closely correlated with total dry biomass, including seed yield. We conclude that our continuous G \times E performance analysis approach for the examination of physiological parameters may be a good practical tool for the Pre-selection of plants with improved EWU in specific environments.

Poster presentations:

Comparative phenotypic analysis of wild and cultivated sunflower for improved crop resilience

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The collection of wild annual and perennial sunflowers in the Institute of Field and Vegetable Crops is one of the largest sunflower collections in Europe. The first hybrids resistant to *Phomopsis helianthi* in the world have been created crossing cultivated sunflower genotypes with wild relatives from this collection. Comparative phenotypic analysis of wild sunflower species, along with a parallel analysis of the same traits in cultivated sunflower could enable identification of morphological parameters of various plant organs that could be useful tools for detection of genotypes tolerant to different environmental stresses.

We have analyzed the morphological specificity of the plant organs, as well as the molecular diversity of 7 annual, 21 perennial wild sunflower species and 19 genotypes of cultivated sunflower, with the aim to find out if observed characteristics have an effect on seed germination and nutrient use efficiency in drought stress conditions. Furthermore, we have carried out comparative morphology of various vegetative organs of tested *Helianthus* species in order to determine phenotypic indicators of drought tolerance. Preliminary results showed that there is significant phenotypic variability in observed morphological traits between tested genotypes indicating that some of them could be useful in pre-breeding and breeding for increased drought tolerance and identification of genotypes that could be used in sunflower crop improvement.

Biochemical and molecular basis of the drought tolerance in field pea

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Drought and osmotic stress as a result of drought lead to oxidative stress and creation of a lot of reactive oxygen species (ROS). Plants have developed antioxidant defense mechanisms in order to neutralize the damaging effects of ROS. The drought stress experiment was conducted on seven selected cultivars of field pea in control conditions (phytotron chamber). The plants were irrigated at the optimal soil water content until the third pair of leaves began emergence, after which soil drought was induced by a termination of irrigation up to 18% (moderate drought), and 9% substrate water content (severe drought). Control plants were irrigated at the optimal level of soil water regime (36% substrate water content).

The activity of antioxidative enzymes: superoxide dismutase (SOD), ascorbate peroxidase (APx) and glutathione reductase (GR) was measured spectrophotometrically. Absciscic acid (ABA) concentration was measured by the Elisa method. The changes in gene expression of antioxidant enzymes (*Cu/Zn SOD*, *cAPx* and *GR*) and three genes of aldehyde oxidase (*PsAO1*, *PsAO2* and *PsAO3*), which participates in ABA biosynthesis, were analyzed. Total RNA was isolated from stressed and non-stressed plant leaves. The gene expression levels of antioxidative enzymes and *PsAO* genes were examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. *Arabidopsis* 18S rRNA was used as internal control. The activity of antioxidant enzymes was changed in all pea cultivars under osmotic stress compared to control. However, different enzymes were activated in the tested cultivars, which might indicate a different degree of tolerance. In the tolerant cultivar Trezor, the mechanism of eliminating ROS is based on the constant increase in activity of all three enzymes, while their activity is decreased in the sensitive cultivar Javor. The results of the analysis of gene expression coding for antioxidant enzymes (*Cu/ZnSOD*, *cAPx*, *GR*) showed that the reaction of the tested cultivars to ROS, created under drought stress, was the result of increased expression of the tested genes in tolerant cultivars, i.e. decreased expression in sensitive cultivars. The stomatal reactions also differed between cultivars, but only under moderate drought conditions, while all cultivars reacted by the faster stomatal closure under severe drought. The correlation between ABA in leaves and changes in stomatal conductance revealed that stomatal conductance in cultivar Dukat was most affected by the accumulation of ABA in leaves (10 fold increase) and that this reaction could be explained by "chemical signals" of drought. Contrary to the previous, the lowest stomatal sensitivity to increased concentration of ABA (2,5 fold increase) in cultivar Javor indicates the "hydraulic signals" of drought. Results obtained in the study of pea cultivars confirmed expression of three genes (*PsAO1*, *PsAO2* and *PsAO3*) needed for the synthesis of enzyme aldehyde oxidase key for ABA biosynthesis. As the expression of gene *PsAO3* had constantly been increased under drought conditions, it could be concluded that this gene is of primary importance for pea plants exposed to stress.

The obtained results show that biochemical and molecular analyses for antioxidant enzymes (SOD, APx and GR) can be useful for testing tolerance to drought stress in field pea genotypes.

In vitro mutagenesis as a tool for speeding up conventional plant breeding: a case study with bread wheat

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A drought-tolerant wheat genotype should be identified and improved for the sustainable food security. In the current study, forward genetic screening technique was followed to combine with *in vitro* somatic embryogenesis and gamma ray applications to improve drought tolerance in bread wheat. Drought-tolerant mutant genotypes were efficiently obtained by mutagen treated mature embryo-derived callus masses subsequently inducing them to somatic embryogenesis and then cultivating them in MS media with 15 and 20 gL⁻¹ PEG6000 (as a drought inducer). Later, obtained over five hundred plantlets were segregated up to fifth generation by combining *in vivo* and *in vitro* techniques. SSR markers were used for genotyping fifth-generation candidate drought tolerant mutants and control. To confirm tolerance level of mutants, fifth-generation mutant lines were evaluated under control and stress condition by measuring their agronomic (Flag Leaf Area, Main Shoot Length, Spike Length, Weight of Grains per Spike, Number of Grains per Spike and 100 Grain Weight) and biochemical (Total Chlorophyll, Carotenoid, Proline, SOD, CAT and POX) performances. In conclusion, this study represents a significant advantage in terms of time-savings (i.e. more than nine months), greenhouse space and work during the generation of mutant plant populations.

Marker associated analysis of drought tolerance in F₂ segregated population derived from reciprocal cross of Sagittario x M₆ Sagittario derived drought tolerant mutant(s)

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Mutations change in plant architecture play a significant role in adaptation to stress conditions. Thus, mutants are valuable resources for genetic variations in crop improvement. Historically the use of mutagenesis in breeding has involved chose the right mutagen, treat the seeds or vegetative organ parts of plant with mutagen for obtaining mutant population, the selection of individual mutants with improved traits and incorporate these mutants into breeding programs. The novel genetic variations obtained from either spontaneous or induced mutants using physical or chemical mutagens can be used in crop genetic analysis and mapping a novel mutation(s) to a well-defined chromosomal region is an important step in this analysis. This study was focused on mapping yield traits in 63 F₂ segregated individuals of Sagittario x M₆ Sagittario derived drought tolerant mutants and 37 F₂ segregated individuals of its reciprocal cross under normal and stress conditions. For this purpose, drought stress was triggered by a 50% decrease in the irrigation water applied to all segregated individuals in a greenhouse. Phenotyping of F₂ segregated individuals were taken by using seven traits (Pollen viability, Flag Leaf Area, Main Shoot Length, Spike Length, Weight of Grains per Spike, Number of Grains per Spike and 100 Grain Weight). As for genotyping of F₂ segregated individuals, genomic DNA of Sagittario and segregated F₂ population was isolated from leaves using 2x CTAB extraction buffer. The SSR markers were selected based on previously QTL studies related to drought stress on bread wheat. The preliminary results of this study demonstrated that Single Marker Analysis showed significant association of three SSR markers with pollen viability, weight of grains per spike, number of grains per spike and 100-grain weight.

Drought priming effects on alleviating later damages of heat and drought stress in different wheat cultivars

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The ongoing change in climate; in particular the increase of drought and heat waves episodes are a major challenge in the prospect of food safety. Under many field conditions, plants are usually exposed to mild intermittent stress episodes rather than a terminal stress event. Previous, but limited studies suggest that plants subjected to early stress (primed) can be more resistant to future stress exposure than those not stressed during seedling stage.

In our experiment we aimed to test if repeated mild drought stresses could improve heat and drought tolerance during anthesis heat and drought stresses in wheat cultivars. Two wheat cultivars, Gladius and Paragon, were grown in a fully controlled gravimetric platform and subjected to either no stress (control) or two (P) drought cycles during seedling stage, at three and five complete developed leaves. Each cycle consisted of withholding water until 35% of soil relative water capacity (SRWC) was reached. At anthesis, both batches were then subjected to either a high temperature stress (32/28°C), drought stress (SRWC of 25%), or kept as control treatments. As a result, 6 treatments were established after the high temperature/drought stress for each cultivar.

Gas exchange and chlorophyll fluorescence were used to investigate the physiological performance of plants. Light response curves showed no difference in assimilation for Gladius and a decrease in assimilation for drought non-primed plants in Paragon. Stress treatments in Gladius increased the saturated photosynthetic rate when compared with control plants but no effect of priming was detected to suggest an alleviating effect in wheat plants. Both cultivars showed a decrease in non-photochemical quenching (NPQ) and an increase in the actual photochemical efficiency of PSII (Φ_{PSII}) in primed plants during heat stress. While Gladius drought primed plants showed no difference, a tendency of increase in NPQ and decrease of Φ_{PSII} was observed in primed plants of Paragon during drought stress. Results show a difference in behavior between cultivars, Gladius performed as both a heat and drought tolerant cultivar, while Paragon was perceived as heat and drought sensitive cultivar.

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Determination of radical scavenging activity and antioxidant power of *Brassica napus* under salt stress

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Salt-tolerant plants have developed various physiological mechanisms that provide tolerance to salinity stress. This study was designed to examine the antioxidant and free-radical scavenging activities of three oilseed rape cultivars (Banacanka, Kata, Zlatna) under salinity stress. The seeds were sown in the Petri dishes with sterile sand and moistened with different concentration of NaCl solution (0, 100, 150 i 200 mM). The Petri dishes were placed in germination chamber with a temperature of 20<=>30°C. After 24h, the seed samples were taken. Further, the seedlings were regularly watered during 7 days with the same amount of water or NaCl solution. After that period, the antioxidant potential elevated under salt treatment separately in the seed and seedling shoot using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) assay. DPPH is a stable organic radical and the capacity of biological reagents to scavenge the DPPH radical can be expressed as its magnitude of antioxidation ability. The reactive rate and the ability of the radical scavenger depend on the rate and the peak value of disappearance of the DPPH. In the FRAP assay, the antioxidants ability to reduce a ferric complex to the ferrous form is measured. DPPH scavenging of seedling shoot was increased in a salt stress-dependent manner compared to control, while this trend could not be noticed in the seed. The seed extract was capable of neutralising the DPPH free radicals via hydrogen donating activity by 43.17, 44.33, 37.77 and 40.17% at concentrations of 0, 100, 150 and 200 mM NaCl, respectively. DPPH scavenger activity of the seed was the highest at the lowest stress level in all tested cultivars. In the cultivars Banaćanka and Jasna, the lowest values were found at 150 mmol/l, while in the Zlatna cultivar the lowest values were observed at a concentration of 200 mmol/l NaCl. The ferric reducing antioxidant potential of the seedling shoot extracts was in the range of 28.63 to 46.8 in the seed and of 1.14 to 4.38 FRAP units. This reducing ability may be directly related to the content of phenols, tannins and flavonoids and their ability to neutralise free radicals. It is known that the oilseed rape seed contains higher amounts of phenol, which can explain the results obtained that show higher FRAP values in the seed than the seedling shoot after 7 days. Also, in the seed extract of Jasna and Zlatna cultivars, the highest values of FRAP were at the lowest concentrations of NaCl (100 mM), which may indicate that lower amounts of NaCl act stimulatingly to activation of an antioxidant defence system. Since it has been proven that an effective antioxidant system can provide better protection against oxidative stress, the total antioxidant activity expressed by FRAP units and DPPH scavenger activity have important practical significance in ranking cultivars and plant species in terms of tolerance to oxidative stress.

A multiple criteria decision-making approach to waterlogging response evaluation in a worldwide collection of *Lathyrus sativus*

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Grass pea (*Lathyrus sativus*) is a high protein content cool-season annual legume crop with extraordinary adaptability to unfavorable environments and resistance to severe diseases. Waterlogging is a major abiotic factor in cool-season grain legumes causing significant yield losses. As other abiotic stresses, waterlogging hampers germination, shoot and root growth, nutrient uptake, and among others, also promote seedling diseases.

There are several literature references on the great level of grass pea waterlogging tolerance, as reported in monsoon waterlogged or rice-growing areas from South East Asia and Africa. However, no detailed study on this subject has been performed yet, giving us the opportunity to identify genetic variation in grass pea waterlogging responses and to understand the associated resistance or tolerance mechanisms. Under scope of the Portuguese QUALATY project, we gather a worldwide collection of 150 *L. sativus* accessions that we will use to fulfil the mentioned purposes.

A survey on diverse experimental stress imposition schemes, timeframe of measurements and current techniques to screen waterlogging tolerance in legumes was conducted. The output constitutes the first step towards the screening of waterlogging responses within this collection. Several phenotyping approaches were forecasted, focused on screening shoot and root biomass accumulation or architecture (reflectance or tomography), on assessing photosynthesis efficiency and stomatal conductance (thermography, fluorescence or gas exchange), microscopic traits (aerenchyma formation or porosity) or biochemical traits (minerals, sugar contents and related enzymatic activities). The use of these approaches will be discussed considering their potential to be applied for high-throughput phenotyping of a large collection of accessions for future association mapping genetic studies, or to a few contrasting accessions for a detailed characterization of the resistance or tolerance mechanisms.

Oxidative stress in black and yellow soybean grown in organic production system

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Dark-coloured soybean seeds contain high concentrations of phenolic compounds that contribute to their high stress resistance. The purpose of this work was to define whether soybean plants developed from black coloured seeds would be protected from (a)biotic stress during seed filling period in organic system of production in comparison to those from yellow seeds. Lipid peroxidation intensity (LP, biomarker of cell degradation and oxidative stress secondary effects, expressed as nmol malondialdehyde or MDA equivalents g⁻¹ fresh weight) and antioxidant capacity (NBT-test, as antioxidant capacity of plant material expressing % of neutralization of reactive oxygen species or ROS produced during oxidative stress) were determined in leaves of soybean plants at the seed filling stage. Plant material tested in this research was soybean [*Glycine max* (L.)Merr.] leaves of two black ('NS Blackstar' and 'NS Pantera') and two yellow ('Fortuna' and 'Galina') soybean cultivars grown in experimental field of Institute of Field and Vegetable Crops, Department for Alternative Crops and Organic Production. Leaves of tested plants had different values of LP intensity (23-52 nmol MDA g⁻¹ fresh weight) and capacity to neutralize ROS (61-88%), however, 'Galina' was highlighted with lower LP intensity (2-fold) and higher antioxidant activity (10-27%) during seed filling period. Since there is no clear difference between black and yellow cultivars in response to oxidative stress during seed filling stage of development, further analyses of biomolecules responsible for colour of seeds could explain which of these compounds participate, among other antioxidants, in oxidative stress defense mechanisms and should propose which cultivar would perform better in organic production system.

Non-invasive analysis of biomass accumulation and geometry of sorghum plants growing under drought

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Recent advances in genome sequencing technologies have shifted the research bottleneck in plant sciences from genotyping to phenotyping. High-throughput plant phenotyping collect a large amount of experimental data about plant surface properties, plant geometry as well functional features that can be used to better understand of plant growth and productivity in stressful environments. The Slovak PlantScreen Phenotyping Unit (SPPU) was used to investigate of growth dynamics and geometrical responses of four sweet sorghum genotypes cultivated in well-watered (WW) and gradual water stress (WS) conditions. For calculation of plant growth analysis parameters (AGR, RGR, NAR) was used the precision calibration of relations between automatically evaluated shoot area from plant RGB imaging and leaf area measured manually by destructive method as well between fresh and dry biomass weight, respectively. Has been observed the significant genotypic differences in above-ground biomass accumulation of plants growing in well-watered as well water stressed conditions. Further, the geometrical description of plant has been done, such as height, circularity (roundness and isotropy), symmetry (eccentricity and RMS) and center distance (compactness and SOL) parameters. Based on data analysis has been observed the genotypic differences in time-dependent dynamics of plant leaf area formation and growth rate (ARG and RGR) as well as NAR. We have identified that oscillation of plant compactness calculated separately for side and top views provides important information about leaves orientation. WS caused significant decrease in value of plant compactness in all genotypes, which can be attributed to a more planophile oriented leaves due to the decrease of the slope angle towards the horizontal plane by the turgor decrease.

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Research adaptation of plant *Nicotiana tabacum* generation T-1 expressing gene *desA* cyanobacteria to cold stress conditions

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Currently, research is very relevant mechanisms of plant resistance to abiotic stress. Adaptation of plants to stress depends, first of all, on the properties of membranes. Together with an increase in the proportion of unsaturated fatty acids in the membrane lipids, the viscosity and fluidity of the membrane lipids increases, the membrane crystallization temperature decreases. Desaturases – are enzymes that contribute to the formation of double bonds in fatty acids and thus convert them from saturated in unsaturated. The *Nicotiana tabacum* T-1 generation plants that carry and express the *desA* gene encoding $\Delta 12$ -acyl-lipid desaturase of cyanobacteria *Synechocystis* sp. PCC 6803, fused in one frame of reading with the genome of the reporter protein of the thermostable lichenase *licBM3* used in the work. The level of accumulation of malondialdehyde and loss of electrolytes after the effect of cold stress was investigated. As control, wild-type *Nicotiana tabacum* plants and *Nicotiana tabacum*, expressing the gene of the bifunctional reporter *gfp:licBM3*, were used. The decrease in the level of accumulation of MDA by 38-41% and reduction of the level of electrolyte loss by 10-12% in plants with genes of desaturases cyanobacteria compared with the control have been revealed.

Assessing genotypic variability in the morpho-functional response of garlic to water availability

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Most garlic commercial varieties have lost their capacity of sexual reproduction except some remaining varieties at the center of origin and growing under specific environments. This is a major limitation for garlic breeding. Therefore, a morpho-physiological based selection approach could be a feasible strategy to improve crop's resistance to abiotic stress namely to drought. Plant morpho-physiological performance is influenced by environmental factors and by the genotype. Thereby, variation among garlic cultivars in response to water deficit is expected. To assess such variability among cultivars a field trial was carried out in Cuenca region (Castilla-La Mancha, Spain) in 2016. Two early maturing cultivars (White Spring and Violete Spring) and three late maturing cultivars (White, Morado de Pedroñeras and Fino de Chinchón) were sown in a split-plot design with two factors (cultivar x water availability).

We measured the pseudo-stem diameter along the trial to evaluate plant growth under different water availability conditions. Leaf stomatal impressions were also analyzed to determine stomatal density and guard cell length in abaxial and adaxial leaf surfaces for each cultivar in each water treatment. At the end of the trial we measured bulb weight and size to evaluate the potential impact of the studied traits on bulb yield. We found significant differences among cultivars and water treatments namely in terms of bulb weight and size. We found also differences in stomatal traits. Complementary traits such leaf gas exchange, chlorophyll fluorescence and other physiological traits are being assessed to ascertain the main morpho-physiological traits implied in garlic phenotype in terms of drought tolerance.

Key words: garlic, stomata, water availability, stomatal density, cell guard length, abiotic stress tolerance, crop yield, water deficit

Changes in root architecture involving drought tolerance in oats

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Drought is one of the major environmental factors determining plant yield. Tolerance to this abiotic stress is a complex phenomenon comprising a number of physio-biochemical processes and also root architecture changes. Our aim is to reveal the morphological changes occurring within the roots that promote drought tolerance in oat. To this aim we employed two oat genotypes, Flega and Patones, previously characterized as highly susceptible and tolerant to drought respectively. Root phenotyping was carried out in both, pots by using destructive techniques, and in intact plants by using rhizotrons. In pots experiments, sampling times were chosen to cover different levels of sRWC: still-sufficient water (8 daww, 55-60% sRWC), mild water deficit (11 daww, 40-45% sRWC), moderate water deficit (14 daww, 30-35% sRWC), high water deficit (17 daww, 20-25% sRWC), and severe water deficit (20 daww; 15-20% sRWC). For the rhizotron study, samples were grown at 90%, 35% and 20% of sRWC during whole experiment. In pots, tolerant genotype maintained similar growth respect its control under drought, and produced high number of roots with less than 0.5 mm of diameter. Rhizotron experiments confirmed these results and highlighted parameters like convex hull area and root system width, where susceptible and tolerant genotypes showed significant differences at 20% of sRWC. This work reveal that early and fine modulated morphological root changes are key factors for promoting drought tolerance in oats.

Ideotype South - Searching for adapted wheat varieties in Mediterranean region of South of Europe

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Climate change impacts can, for example, cause decreasing wheat yields in parts of Europe, namely in the Mediterranean region, due to heat stress and reduced rainfall that affect modifications on crop phenology - crop season will be shorter. A key approach must be the comprehension of the varietal adaptation and effectiveness will be added when studied in cooperation. In this context, a research Program is being developed in Portugal and France designated the Wheat Ideotype South Project, aiming the definition of wheat variety type (ideotype South). This research program is carried out since 2011, by the Portuguese Institute of Agrarian and Veterinarian Research (INIAV), a research institute under the umbrella of the Ministry of Agriculture; the Portuguese farmer's association for cereals and pulses (ANPOC) and ARVALIS - Institut du Végétal, an agricultural applied research French organization financed and managed by farmers. The performance and response of French and Portuguese bread and durum wheat varieties and advanced lines obtained by INIAV are evaluated in different conditions in order to study the interactions between genetics, environment and cultural practices. This research Program uses innovative tools (e.g. genetics of association) and methods (e.g. phenotyping) to defining best-fit traits of a new plant (wheat) ideotype, which enable agricultural systems adaptation to climate changes scenarios, that are expected to occur in the near future (breeding new wheat varieties). The results obtained indicate that the ratio grain quality/yield for wheat spring Portuguese varieties are more interesting during unfavorable seasons (concerning weather). These varieties are less fragile concerning test (volume) weight, an important trait for evaluating adaptation to heat stress. Results shows the good adaptability of the genotypes selected in harsh environments, being test weight an excellent indicator of good sink capacity even if the expression of yield potential might be sacrificed. French varieties suffered a lot under higher heat stress, as it is common in the south Portuguese Mediterranean environment. Another important outcome of this research Program is to promote the recombination between Portuguese and French germplasm in order to obtain genetic variability for adaptation. An intensive artificial crossing program, in each season, is being developed combining both Portuguese and French advanced lines, carrying the desired traits that are being identified by researchers. This network is, also, an important way for the identification of the most suitable germplasm that can overpass future environmental constraints.

Winter wheat agronomic traits as affected by soil cadmium contamination

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Due to anthropogenic pollution soil Cd concentration can exceed permitted limits and that could lead to the production of food that contains high level of Cd in edible plant parts. The aim of this study is to investigate the effect of soil Cd contamination on winter wheat agronomic traits (plant height (cm), spike length (cm), plant weight (g), spike weight (g), number of fertile spikelets per spike, number of sterile spikelets per spike, number of grains per spike, grain weight per spike (g) and 1000 kernel weight (g)) as well as Cd concentration in a different plant parts in anthesis and full maturity. Experiment was carried out in semi controlled conditions. Based on the results of previous research, ten winter wheat cultivars that had wide range of grain Cd concentration were selected and included in this research. Cultivars were sown in the pots that were filled with soil contaminated with 0, 2 and 5 mg Cd kg⁻¹ soil. Experiment was set up according to completely randomized block design with four replicates. First sampling was carried out in anthesis when ten randomly chosen plants were cut from the pot and samples of root, stem, flag leaf, leaves and spike were collected. In the second sampling that was carried out in full maturity, samples of straw, leaves, glumes and grain were collected from ten randomly chosen plants from each pot. Cd concentration in samples was determined by ICP-OES technique. Measures of descriptive statistics were calculated for all examined traits. Effect of examined treatments was tested with factorial analysis of variance ($p < 0.01$) and where necessary, differences between levels of examined treatments were assessed by Tukey's HSD test ($p < 0.01$). Statistical analysis was carried out in SAS 9.4. for Windows and SAS Enterprise Guide 7.1. As results showed, plant height ($F(18) = 3.5$; $p < 0.01$), spike length ($F(18) = 2.1$; $p = 0.013$) and number of sterile spikelets per spike ($F(18) = 2.3$; $p < 0.01$) were under significant cultivar x Cd interaction effect. Plant weight ($F_{\text{cultivar}}(9) = 5.4$; $p < 0.01$ and $F_{\text{Cd}}(2) = 14.8$; $p < 0.01$) and spike weight ($F_{\text{cultivar}}(9) = 4.8$; $p < 0.01$ and $F_{\text{Cd}}(2) = 9.3$; $p < 0.01$) were under significant effect of cultivar and Cd treatment, but there was no evidence of significant interaction effect. Furthermore, there was a significant difference in number of grains per spike ($F(9) = 2.4$; $p = 0.019$) and 1000 kernel weight ($F(9) = 6.5$; $p < 0.01$) between cultivars. Soil Cd contamination significantly increased Cd concentration in all examined plant parts in anthesis and full maturity. In general, highest Cd concentration in all examined plant parts has been determined under highest Cd treatment (5 mg Cd kg⁻¹ soil). In anthesis, highest average Cd concentration was determined in roots (29.5 mg kg⁻¹), followed by leaves (4.92 mg kg⁻¹), stem (3.71 mg kg⁻¹), flag leaf (1.63 mg kg⁻¹) and spike (0.97 mg kg⁻¹). In full maturity, average Cd concentration under highest Cd contamination was as follows: straw (4.28 mg kg⁻¹), leaves (3.69 mg kg⁻¹), glumes (1.49 mg kg⁻¹) and grain (1.34 mg kg⁻¹). Additionally, examined cultivars differs significantly in Cd concentration in examined plant parts on uncontaminated and Cd contaminated soil, indicating on genotype specificity regarding Cd accumulation in different plant parts.

Early changes in physiological parameters after *Trichoderma*-Tomato interaction in water stress conditions

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Investigations on the effect of *Trichoderma brevicompactum* on plants are limited in comparison to other *Trichoderma* species, as they are known for the production of phytotoxins. However our previous results indicate that they have beneficial effect on plant water status in tomato grown under increasing water deficit, up to twelve days after the treatment. The results on early changes of several non-destructive parameters of tomato plantlets in response to *T. brevicompactum* and water stress were investigated in this report.

Tomato plantlets (*Solanum lycopersicum* Mill. cv. Ailsa Craig) were grown under controlled conditions with optimal water supply (75% soil water capacity) until the 4th leaf developmental phase. Plants were subjected to three treatments: cessation of watering, addition of the *T. brevicompactum* SZMC 22661 spore suspension (8×10^6 CFU) to the root zone, and combination of both treatments.

Several parameters were measured by non-destructive probes: soil water content (*Theta* probe; *Delta-T, Cambridge, U.K.*), content of chlorophyll and epidermal flavonols (Dualox Scientific sensor; Force A, France) and stomatal conductance (porometer; *AP4, Delta-T, Cambridge, U.K.*). Leaf water potential was determined by pressure probe. Measurements were performed 2, 6, 24 and 48 hours from the onset of treatments.

Decreased stomatal conductance and leaf water potential were observed as early reactions (up to 6h) to *Trichoderma*-tomato interaction. However 48h after the beginning of treatment, plants that experienced water stress and *Trichoderma* presence exhibited higher stomatal conductance and leaf water potential in comparison with droughted plants without *Trichoderma* treatment. The observed increase of flavonol content, 24h after the onset of treatments was significantly smaller in *Trichoderma* treated plants. The beneficial effects of *T.brevicompactum* to plant water status are discussed in terms of previous results and further investigation.

Two mitogen-activated protein kinases MPK12 and MPK4 are essential components of CO₂-induced stomatal regulation

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Stomata are pores in epidermal plant tissues, which are formed by surrounding guard cells and control gas exchange in plants. Stomatal regulation is under intense research; however, there are still gaps in understanding molecular details of guard cell signalling. Previous research showed that the *Arabidopsis* protein kinase HT1 regulates CO₂-induced stomatal movements. HT1 is classified as a Raf-like protein kinase. Our recent studies have identified mitogen-activated protein kinase MPK12 as an interactor of HT1 that inhibits HT1 kinase activity.

The objective of this research was to study signalling cascade(s) that involve MPK12 and HT1. As MPK12 is a protein kinase we analyzed the role of relevant mitogen-activated protein kinase kinases (MKK1, MKK2, MKK3, MKK4, MKK7, MKK9, MKK10). Experiments with plant lines carrying mutations in these genes showed that none of the mentioned kinases influenced CO₂-induced stomatal movements.

We also examined protein kinases (MPKs) with high guard cell RNA expression levels, namely MPK4, MPK9, MPK11, and MPK19. MPK4 and MPK11 are phylogenetically close to MPK12. However, MPK4 interacted with HT1 in both yeast two-hybrid interaction test and bimolecular fluorescence complementation test, while MPK11 did not.

We found that among mentioned MPKs only plant lines with impaired MPK4 demonstrated somewhat impaired stomatal responses to changes in CO₂ concentration, and had less obvious impairment on ABA response.

Further studies revealed that transgenic plants lacking both MPK4 and MPK12 expression in guard cells completely abolished CO₂-induced stomatal movements, while still responded to ABA treatment.

We have identified MPK4 as a new component involved in CO₂-induced stomatal movements and have showed functional redundancy of MPK12 and MPK4 proteins in plant. Obtained results suggest that MPK4 and MPK12 play an essential role in CO₂-induced stomatal regulation whereas several other MPKs such as MPK9, MPK11, and MPK19, do not.

Obtaining new data regarding MPK4, MPK12 and HT1 interaction networks can give us tools for agronomic breeding and improving of crop production via directional regulation of plant growth, development and stress resistance.

Time-course analysis of stomatal conductance, chlorophyll content and fluorescence in barley under drought stress

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Drought is a condition in which crop lacks water due to reduced rainfall or insufficient irrigation. Drought decreases yield by reducing germination and later, accelerating leaf senescence. Understanding of drought response and tolerance of crops and therefore selection requires plant phenotyping. Water status (leaf conductance) and chlorophyll status (amount and fluorescence) are indicators of plant health and performance and appropriate parameters for phenotyping. In this study, 5-day-old barley (*Hordeum vulgare* cv. Carina) plants were subjected to progressive drought through water withholding for 22 days. Control plants were irrigated regularly to maintain soil water content (SWC) of 65%. Control and drought plants were monitored every 2 days for SWC, leaf water content (LWC), stomatal conductance (gS), chlorophyll content and fluorescence. Under drought conditions, early closure of stomata helped maintain LWC and chlorophyll content and decreased yield of PSII photochemistry.

PSII reaction centers did not close and excess energy was dissipated by down-regulation. However, under well-watered conditions, non-regulated energy dissipation increased in later stages. Drought-induced changes in all parameters except gS were independent from age. gS was the first and most affected feature under drought conditions. gS and chlorophyll fluorescence were strongly correlated with LWC. Regulation of stomata and photo-protective processes may be the prominent mechanisms of drought response and tolerance.

Using supervised machine learning to explore *Vicia faba* adaptations to agro-climatic

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Faba bean (*Vicia faba* L.) is an important source of protein but breeding for increased yield stability and stress tolerance is hampered by the scarcity of phenotyping information. Because comparisons of cultivars adapted to different agro-climatic zones improve our understanding of stress tolerance mechanisms, the root architecture and morphology of 16 pan-European faba bean cultivars were studied at maturity. Different machine learning (ML) approaches were tested in their usefulness to analyse trait variations between cultivars for future breeding. A supervised, i.e. hypothesis-driven, ML approach revealed that cultivars from Portugal feature greater and coarser but less frequent lateral roots at the top of the taproot, potentially enhancing water uptake from deeper soil horizons and thus drought tolerance.

Osmotic stress responses of *Bradyrhizobium japonicum* strains

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The aim of this study was to investigate osmotic stress tolerance ability of *Bradyrhizobium japonicum* strains. The research was performed with 30 *Bradyrhizobium* strains from the collection of Institute of Field and Vegetable Crops Novi Sad, Serbia. *Bradyrhizobium* strains were tested on osmotic stress tolerance induced by supplementing the liquid yeast extract mannitol (YEM) medium with four different concentrations of polyethylene glycol (PEG) 6000. Polyethylene glycol (PEG) is more widely used than other substances (sugars or salts), because of its ability to decrease external water potential by binding water molecules in solute, without penetrating the cell wall. Strain precultures were grown in 100 ml liquid YEM medium, with 150 rpm at $28 \pm 2^\circ\text{C}$ for 72h. To test strain osmotic stress tolerance, 1 ml of preculture was transferred in 50 ml YEM medium supplemented with four different PEG concentrations 9%, 17%, 24%, 36% (w/v), and in YEM medium without the added PEG (control treatment). Test cultures were incubated for 72h ($28 \pm 2^\circ\text{C}$, 150 rpm). Serial dilutions were prepared from the test cultures and spread onto an agarized YEM medium. Growth pattern was recorded by plate counts over colony forming units (CFU ml⁻¹) five days after incubation at $28 \pm 2^\circ\text{C}$, and expressed as log₁₀ CFU ml⁻¹. The number of colonies formed by control culture was set to 100% of growth for each bacterial strain (represents control treatment) and the number of colonies formed by stressed cultures was normalized accordingly.

In control treatment, log₁₀ CFU ml⁻¹ ranged from 3.31 to 20.40 in strains BJ6 and BJ26, respectively. Among the strains, significant reaction differences to osmotic stress were obtained. Decrease in water potential, due to the added PEG, resulted in significant growth reduction of all tested strains, and all *B. japonicum* strains showed lower growth in the presence of 9% PEG. Osmotic stress, induced by 24% PEG, suppressed strains persistence to a very different extent, and the number of formed colonies ranged from 7.22 in strain BJ31 to 0.50 in strains BJ27, BJ28, BJ32. The growth reduction of strain BJ11 was the least affected in the presence of 36% PEG (reduced by 20.6%). Strain BJ27 showed lowest tolerance to osmotic stress, and comparing to control, number of colonies was reduced by 91.1%.

Desiccation stress specifically induces expression of *Bradyrhizobium japonicum* genes responsible for protective mechanisms such as proteins involved in polysaccharide synthesis, protection of the cell membrane, repair of DNA damage, oxidative stress responses. These results indicate that different strains of the same bradyrhizobial species could have specific adaptive response to desiccation and differ in genetic potential that provides stress resistance.

ABA synthesis in guard cells or phloem controls stomata, but OST1 is the key for VPD response

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Guard cells shrink and close stomatal pores when air humidity decreases, i.e. when the difference between the vapor pressures of leaf and atmosphere (VPD) increases. The role of abscisic acid (ABA) in VPD-induced stomatal closure has been studied using ABA-related mutants that respond to VPD in some studies and not in others. The importance of ABA biosynthesis in guard cells versus vasculature for the whole-plant stomatal regulation is also unclear. Here we show that *Arabidopsis* lines carrying mutations in different steps of ABA biosynthesis as well as pea wilted and tomato flacca mutants had higher stomatal conductance compared to wildtype plants. To characterize the role of ABA production in different cells, we generated transgenic plants where ABA biosynthesis was rescued either in guard cells or in phloem companion cells of an ABA-deficient mutant. In both cases, the whole-plant stomatal conductance, stomatal index, stunted growth phenotype and leaf ABA level were restored to wildtype values, pointing to the redundancy of ABA sources and to the effectiveness of leaf ABA transport. All ABA-deficient lines closed their stomata rapidly and extensively in response to high VPD, whereas plants with mutated protein kinase OST1 showed no VPD-induced responses. Another strongly ABA-insensitive mutant defective in six ABA PYR/RCAR receptors, responded to changes in VPD in both directions strongly and symmetrically, indicating that its VPD-induced stomatal closure could be hydropassive. We discuss that both the VPD-induced hydropassive stomatal closure and the stomatal VPD-regulation of ABA-deficient mutants may be conditional and depend on pretreatment stomatal conductance.

Guaiacol peroxidase activity in sunflower hybrids seedlings

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Environmental conditions, such as high and low temperatures, drought, UV radiation, increased soil saturation, pesticides, pathogenic infection, can cause oxidative stress in plants. These changes are transmitted from the cells to various plant organs, which cause a large number of physiological changes - decrease of seed germination, resistance of plants to drought and different diseases, which ultimately results in a yield decrease. Peroxidases have an important function in defending plants against the harmful effects of free oxygen radicals and are involved in many processes in plants. During seed germination, metabolic activities such as intensification of the respiration, protein synthesis, nucleic acids occur. These processes have an important function in seedling cells lengthening and completion of germination process. In testing of sunflower seed quality the manifestation of the problem with the occurrence of atypical seedlings is often. In our conditions, the most common problem is the lack of a primary root. The aim of the study was to determine whether the lack of primary root causes an increase in peroxidase activity. The working samples of the fresh plant material needed for the analysis were obtained by untreated NS-H-111, Oliva and Sumo 2 OR hybrids. Applied the standard laboratory methods. For analysis, 10 typical and 10 atypical seedlings were selected, and atypicality was related only to the lack of primary root, while other structural parts were normally developed. Extracts of fresh plant material (separated shoot and root) were obtained by homogenization of 1 g of material with the addition of 10 ml of 0.1 M KH₂PO₄ buffer (pH 7). Supernatant was used as a sample for analysis. The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was determined by the method of transforming guaiacol into tetraguaiacol for 1 minute. The results showed that guaiacol peroxidase activity significantly dependent only of seedling type. In all three examined hybrids guaiacol peroxidase activity was significantly higher at atypical seedlings. Significant differences not found between the organs of the seedling, but it noticed that guaiacol peroxidase activity at hybrids NS-H-111 and Sumo 2 OR was higher at the root, while in the Olive hybrid the higher enzyme activity registered in the shoot.

Monitoring of plants by high-throughput phenotyping facility in hydric stress context

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The Roullier Group's Global Research & Development Centre was opened in the heart of Brittany one year and half ago. The Centre will carry out research on microbiology, Plant and Animal Nutrition. On a 2-hectare site with 8000 m² of building, 1200 m² of labs and 800 m² of greenhouses, this vast development will serve as an incubator for future nutritional products. Innovation represents a constant and continuous challenge and mobilizes research teams who work in strict cooperation with Universities and Research centres in different countries. The research team in the field of Plant Nutrition operates in several directions like the study of biotic and abiotic stresses, plant metabolism or soil/roots interaction. We are also interested by a lot of plant parts such as shoot, root, seed or sap flow. In conjunction with our laboratory analysis, the image-based methods are mostly used in our internal and collaborative projects. As part of the new R&D Centre, a high-throughput phenotyping facility was built with a capability of 685 plants. This facility allows non-destructive measurements throughout vegetative development by two imaging cabins equipped by different acquisition systems like colour, infrared and thermal imaging camera. We defined our own image processing pipeline to extract useful information from raw data like morphometric measures, vegetation indexes or foliar temperature. We show herein our results on a hydric stress trial where we monitoring plants with no stress, constant stress and resilience to a periodic stress. A comparison between phenotyping traits and laboratory data allows identifying some correlations for this kind of application.

High-throughput phenotyping of an elite durum wheat association panel reveals differential selection for a root architecture QTL in response to different water regimes

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This study reports the characterization of 183 elite durum wheat (*Triticum turgidum* ssp. *durum* Desf.) for root system architecture (RSA). Plants were grown in controlled conditions up to the 7th leaf appearance (late tillering) using the high-throughput phenotyping platform GROWSCREEN-Rhizo, integrated with automatic root RGB imaging. The following RSA traits were measured: seminal root length, nodal root length, lateral root length, root system convex hull, root system width and depth distribution (twice per week). Measurements of leaf area, leaf number and tiller number were performed twice per week and SPAD measurements were collected twice during the experiment. Root dry biomass and shoot fresh and dry biomass were collected at the end of the experiment. The dynamic collection of the above-mentioned traits allowed us to model root and shoot growth and thus to disentangle the final point phenotypes in simpler and integrative phenes. GWAS with the Illumina 90K SNP assay identified many QTLs for RSA and/or shoot growth traits ($P < 0.0001$). GWAS confirmed a highly significant effect on adult plant root system width due to two major QTLs on chromosomes 6AL and 7AC (Maccaferri et al. 2016). Notably, haplotype frequency of the QTL cluster on chromosome 7AC was associated with root depth, width, specific weight and shoot/root ratio. Notably, shoot/root ratio revealed a strong, contrasting selection pattern between the cultivars released by the rainfed and irrigated breeding programs conducted at ICARDA and CIMMYT, respectively, suggesting an indirect role of RSA features in durum wheat breeding and environmental adaptation. The accessions used in this experiment were previously tested for yield in 15 different environments across the Mediterranean basin at different water and climatic regimes. These results provide valuable insights toward a better understanding and more effective deployment of the RSA QTLome in durum wheat as related to different environmental conditions.

High-throughput phenotyping platforms for screening of root traits on edaphic stress adaptation

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Plant roots are crucial for the water uptake and nutrients from soil. Root systems of major agricultural crops are poorly adapted to soils that mostly have poor water holding capacity and nutrient deficiencies. Root traits, such as deep root systems, increased root density in subsoil, increased root hair length and density may contribute to enhanced water and nutrient uptake. However, characterizing root system architecture (RSA) is challenging, as roots are difficult objects to observe and analyse.

Many new technologies have been developed to characterize crop root system architecture. High-throughput phenotyping platforms (HTPP) developed for plants under controlled conditions are used to provide reliable estimates of phenotypic traits. Large-scale phenotyping of root-related traits remain the most important challenge in translating physiological and genetic advances in understanding the role of root systems in improved adaptation to abiotic stress and enhanced productivity. Recently, the advent of large-scale phenotyping, non-destructive root imaging and analysing, and root structure-functional modelling technologies enables our understanding of the in sight of root-soil interactions and adaptation to edaphic stress.

In this review, we discuss recent developments low-cost high-throughput phenotyping platforms for screening of root traits on edaphic stress adaptation and critically assesses approaches available for high-throughput phenotyping of root traits at both laboratory and field conditions.

Keywords: high-throughput phenotyping platforms, root traits, edaphic stress.

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Application of fast non-invasive methods for phenotyping the responses of lettuce plants to drought and mycorrhizal inoculation

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The symbiosis of plant roots with arbuscular mycorrhizae fungi are known to alleviate the level of stress from drought in some vegetable species, such as lettuce. In addition to biomass production, the important issue is also the content of nutritionally valuable compounds. In leafy vegetables, the attention is recently focused mostly on flavonoids, the strong antioxidants with a key role in plant defense mechanisms in reaction to various stressors. As the flavonoids in lettuce are dominantly located in the epidermis, the dynamics of flavonoids can be efficiently screened by the non-invasive fluorescence-based method using the portable non-contact sensor. The purpose of this study was to evaluate the effect of mycorrhizae fungi of the genus *Glomus* on the production of biomass of lettuce plants *Lactuca sativa* L. in normal and drought conditions, and on dynamics of flavonoids in the leaves of these plants. Our results indicate that mycorrhiza had a positive effect on production of the lettuce plants under drought stress, with different acclimation mechanisms to drought in different cultivars of lettuce. We observed also unequal patterns of flavonoid content dynamics in relation to genotypes and inoculation treatments. Our results confirm that the fast, non-invasive fluorescence sensor can be used for phenotyping of plant-stress interactions as well as plant-microbial interactions, with applications in vegetables breeding aimed at production and qualitative traits.

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