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New sensing technology for affordable phenotyping in plant breeding

Results from the ECOBREED and other ongoing research projects

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Obituary: Tamás Lelley (1942-2023)

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Tamás Lelley, cytogeneticist, researcher and academic teacher, died on 1st January 2023 after a serious illness at the age of 81.

Tamás Lelley was born on 10th April 1942 in Nitra, Slovakia. Tamás completed secondary school in Eger, Hungary, and studied from 1960 to 1964 in Gödöllő. In 1967, he received his doctorate with a thesis on the karyotype analysis of triticale supervised by Prof. Barna Győffry. Tamás Lelley received his habilitation in 1982 at the Georg-August University of Göttingen, Germany, with a thesis on the genetic interaction between rye and wheat genomes in triticale under mentorship of Prof. Gerhard Röbbelen.

Tamás' career led him from the Academy of Sciences in Budapest via a stopover in commercial plant breeding at the breeding company Gebrüder Dippe to the University of Göttingen, where he worked as a scientist from 1970 to 1991. Scientific stays took him from 1976 to 1977 to the University of Winnipeg, Canada, and from 1991 to 1992 to the John Innes Centre in Norwich, UK. Finally, Tamás Lelley moved in 1992 to BOKU University in Vienna where he worked from 1994 until his retirement in 2009.

Tamás Lelley had contact with plant breeding already as a child: his father János was a widely recognized wheat breeder in Hungary and the world. Tamás Lelley gained international reputation as a cytogeneticist through his work on triticale and on wheat-rye chromosome translocations. He was one of the researchers who took up genetics and heredity as a new research topic in Hungary in the 1960s, which was considered inappropriate for long time in socialist Eastern Europe in the heydays and aftermath of Lysenkoism. Tamás Lelley was always curious about new scientific developments. For example, he did pioneering work on triticale, a crop that had hardly been worked on in the 1980s. From the 1990s onwards, he devoted his work more and more to molecular genetics, however, without giving up his cytological talent. He was a talent at the microscope and as teacher, conveying his fascination for chromosomes to his students like no other. He was one of the first to decipher the structure of wheat and rye chromosomes, for which he used the then novel differential chromosome banding technique. During his last period of work in Austria, he turned his attention to new research fields, *e.g.* the resistance breeding of Styrian oilseed pumpkin.

Tamás Lelley published over 170 scientific articles, more than half of them in renowned international journals. For many years, he was a member of the editorial board of Euphytica. Under his supervision, 12 doctoral students received their doctorates, 6 in Göttingen and 6 in Vienna. He was a patient and tireless supporter of young scientists. Many graduates who completed their master thesis or dissertation under his supervision hold today leading positions in commercial breeding and research. His level-headed nature, tireless drive, scientific correctness and last but not least friendly manner make him a lasting academic role model for his students and colleagues.

Tamás was a regular participant at the annual conference in Raumberg-Gumpenstein. Saatgut Austria and the „Gumpenstein community" will always remember Tamás Lelley.

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Digital-agriculture: How to turn crop sensing data into intelligence for improving production

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Abstract

Potato breeding has undergone significant evolution in the past century and a half, adapting to changing consumer preferences and industry demands. Initially focused on increasing yield and disease resistance, breeding efforts have diversified to encompass traits like crop development, maturity, compact growth etc. (de Jesus Colwell *et al*., 2021). Field trials assessing variety characteristics have become integral to breeding programmes, aiding in the selection of high-performing, resilient varieties with desirable quality traits. Such advancements in yield are attributed not only to breeding efforts but also to improved agronomy and crop management practices among farmers. Understanding consumer trends in potato consumption is vital for breeders to tailor variety selection and cultivation practices to meet market demands effectively (Devaux *et al*., 2021). Moreover, while breeding can be conducted without detailed genetic information, analysing target breeding traits accelerates and enhances the breeding process. Key traits such as morphological indicators, disease resistance, and yield parameters are crucial for ecological and evolutionary studies, necessitating standardised measurement protocols. Climate change impacts on potato production, as highlighted in studies, emphasise the need for adaptive breeding strategies and variety selection to ensure resilience and productivity under changing environmental conditions (Obidiegwu *et al*., 2015).

Furthermore, the integration of high-throughput phenotyping platforms and innovative screening techniques offers promising avenues for variety selection. Vegetation indices derived from multispectral unmanned aerial vehicle (UAV) imagery have emerged as valuable tools for monitoring plant health, vigour, and stress levels. Employing remote sensing techniques for disease detection, particularly using UAVs and machine learning models, presents efficient alternatives to labour-intensive field-based approaches (Slater *et al*., 2017; Waiphara *et al*., 2022). These advancements pave the way for improved disease management and sustainable potato production practices. The overarching goal of our research is to refine classification approaches for grouping disease-prone potato crops based on vegetation indices and growth stages, facilitating accurate disease monitoring across multiple years.

The field experiments were carried out at Nafferton Farm, Newcastle University, UK, as a part of the ECOBREED project, as a multi-environment potato varietal trials. Using a randomised complete block (RCB) design with three replicates, 64 potato varieties, prevalent in Europe, were assessed. Various field characteristics were evaluated, including morphological and phenological traits i.e. canopy height, canopy cover, days to flowering, and vegetation indices like NDVI and NDRE. Canopy height measurements were taken post-flowering, and disease assessments focused on fungal pathogens like *Phytophthora infestans* and viruses. Yield assessments were conducted to gauge potato variety performance. Aerial data collection involved UAV flights using both multi-rotary wing and fixed-wing platforms, equipped with multispectral and RGB sensors, respectively and data processing involved extracting canopy parameters from multispectral imagery using R Studio. Data analysis included robust least squares regression for yield prediction, using evaluation metrics such as RMSE and R2. In the first instance, this study evaluated the structured exploration of data extraction methods across different models and software aimed to identify the most reliable method for the potato study, emphasising accuracy, precision, and consistency across datasets and years. This approach allows for thorough comparison and selection of the optimal method based on specific application requirements.

The semi-automated recognition of plants (plant count) achieved an overall accuracy of 82.88% (Masked Auto-Encoder MAE = 0.216) during the initial growth stage UAV flight, decreasing to 61.26% (MAE = 0.523) and 57.37% (MAE = 0.558) during subsequent flights due to increased plant size and potential confusion with weeds. Illustration of plant count and further data evaluation is represented (Fig. 1). Hence, the initial flight was utilised for plant count determination, considering the software's reduced accuracy with larger plants and potential weed presence. Disease prediction using a random forest classification approach based on vegetation indices from multispectral UAV-based imagery yielded an accuracy of 78% during the tuber filling stage in 2021. However, early season inspection in 2020 yielded less accurate results due to healthier crops, emphasising the importance of growth stage timing for disease assessment. Yield prediction models incorporating canopy parameters like canopy area and average plant height achieved varying accuracies across different years. Disease severity classification via random forest achieved
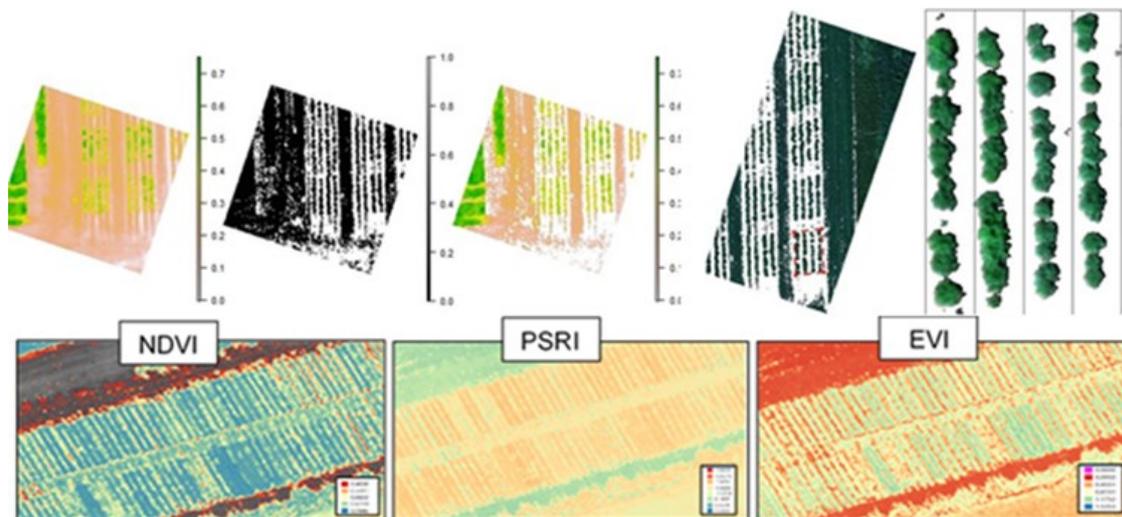
Figure 1 Illustration of plant count data collection and comparative indices generation used in this study.

accuracies of 63% in 2020, 74% in 2021, and 65% in 2022, indicating improved performance with more vegetation indices available. From a weather perspective, temperature variations across years influenced yield patterns, with some varieties showing significant fluctuations. While higher yields correlated with intermediate relative humidity, further investigation is needed, especially concerning specific variety responses and disease incidences. This study's comprehensive analysis underscores the complexity of plant growth dynamics, disease prediction, and yield estimation in potato crops, highlighting the importance of utilising advanced techniques like random forest classification and vegetation indices from UAV imagery. Ongoing refinement and validation of models are essential to enhance the accuracy and reliability for practical agricultural applications.

Keywords

High throughput phenotyping · machine learning · *Solanum tuberosum* · spectral analysis

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Affordable AI driven plant phenotyping: An agricultural engineer's perspective

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Abstract

The agricultural sector is currently facing a convergence of demanding challenges, primarily driven by a shortage of labour, the impacts of climate change and increasing environmental protection standards. These challenges are acting as catalysts, accelerating advancements in agricultural technology. Central to this technological revolution is the role of digitalization, which is not only reshaping the landscape of modern farming but also emphasizing cost efficiency in its implementation. A significant factor in this evolution is the adoption of standard components from consumer electronics and the automotive industry. By integrating technologies initially developed for these sectors, the agricultural industry is able to leverage sophisticated digital solutions at a more affordable cost.

The technologies emerging from the advancements in agricultural engineering and digitalization have a particular potential for plant breeding, especially in the field of plant phenotyping. Low-cost sensors and their data interpretation based on AI methods play a special role here (Riegler-Nurscher *et al*., 2020). These sensors include RGB, multispectral, hyperspectral and thermal cameras as well as ultra-sonic sensors. Light detection and ranging (LIDAR) and depth cameras or radar and fluorescence sensors are also becoming increasingly affordable. All these sensors provide spatial and radiometric information. When focusing on spatial information, convolutional neural networks in particular and increasingly

also vision transformers can be used for image analysis. Different computer vision problems can be solved, as shown in Figure 1.

These methods are based on large amounts of data, which poses challenges, particularly in terms of data recording, data labelling and different non-standardized data sources. In order to reduce the data problem somewhat, techniques such as image augmentation (rotation, brightness, etc.), photorealistic image rendering and, increasingly, generative AI are utilized. For robust applications, possible edge cases should also be covered in the data. However, the issues of robust and standardized validation of these models are still open.

The applications in plant phenotyping, but also in agriculture in general, are very diverse. They range from plant and plant organs detection (Weyler *et al*., 2023) as well as plant height measurement to detecting diseases and stress and even pest and small insects (Feuer *et al*., 2023). The smaller the structures, the higher the resolution required and the greater the volume of data generated. Handling these data streams will remain a major challenge in the near future.

Particular attention must be paid when using unmanned aerial vehicles (UAVs) for monitoring crops. In addition to all legal issues, logistical issues and the desired flight altitude and resolution must be determined in the flight planning phase. In the subsequent flight, ground control points or real time kinematic (RTK) GPS must
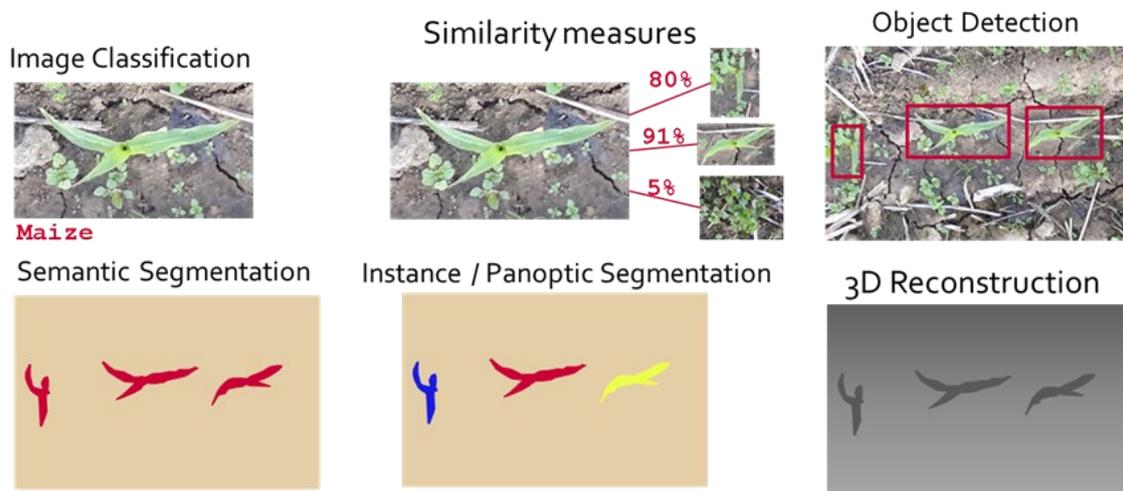
Figure 1 Common computer vision problems in agriculture

be used as required to ensure precise geo-referencing during image stitching or mono plotting.

New trends will shape AI driven agriculture in the near future. The rapid advancements in edge computing hardware in agriculture is enabling real-time processing of data directly on farm machinery. This technology enhances precision farming by allowing immediate decision-making, such as adjusting planting or harvesting strategies on the fly. Active vision systems in agriculture can dynamically adjust to monitor crop health and growth. Unsupervised and semi-supervised learning techniques are valuable in agriculture for analyzing large amounts of unlabeled data collected from fields (Shorewala *et al.*, 2021). They can help in identifying patterns related to crop parameters without extensive manual data labelling. Reinforcement learning-based robot systems will also find their way into agriculture. In general, the combination of AI and robotics will have a lasting impact on agriculture in the near future.

Keywords

Agricultural technology · artificial intelligence · plant phenotyping

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Practical strategies for automated phenotyping: from raw UAV data to multispectral time series for machine learning applications

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Abstract

This study addresses the transition from raw unmanned aerial vehicle (UAV) acquisitions to a multispectral image time series for machine learning applications in precision agriculture. Traditionally reliant on manual labour, wheat breeding and phenotypic assessment suffer from subjectivity and inefficiency. To harness efficient machine learning methods, accurate datasets are essential. Our objective is to standardise procedures, enhance accuracy and facilitate scalability when creating those datasets. We realise a study on the experimental field at Obersiebenbrunn, Austria, managed by Saatzucht Edelhof. Using a custom hexacopter equipped with a multispectral camera, we acquire image data which are subsequently processed by using Pix4Dmapper to generate large orthomosaics. To be organised into comprehensive data, the time series of images are aligned with expert-assessed ground truths for machine learning training. We address challenges such as data processing, experimental design and geolocation accuracy, including evaluating resampling algorithms. Our benchmark justifies the use of bicubic resampling for balancing computational efficiency and image quality. This study contributes to advancing machine learning applications in remote phenotyping and precision agriculture, offering insights into overcoming technical challenges and enabling standardised, scalable solutions.

Keywords

Abiotic stress · machine learning · multispectral imaging · UAV remote sensing · wheat phenotyping

Introduction

Wheat breeding relies on manual labour. Biotic and water stress, as well as other phenotypic traits, are typically assessed by experts using scoring methods. Despite the existence of long-established national and international scoring systems (Saari *et al.*, 1975; Lancashire *et al.*, 1991), the accuracy of the measurements largely hinges on the breeder's skill and judgment. To tackle this issue and establish standardised procedures, machine learning emerges as a powerful solution. Machine learning algorithms can accurately predict phenotypic traits (Rasmussen *et al.*, 2016; Tantalaki *et al.*,

2019; Sharma *et al.*, 2020) offering advantages such as easier harmonisation and updates, centralised execution and ultimately, greater uniformity in measurement quality. Moreover, specific wheat-related characteristics like protein content or dry biomass often require destructive sampling, specialised equipment and expensive infrastructure, which makes their acquisition impractical. In contrast, remote sensing-based measurements are non-destructive by nature. Remote sensing uses sensors mounted on aircraft or satellites to gather and analyse electromagnetic radiation reflected from the target object or area, thus diminishing the need for specialised agricultural equipment on-site.

However, remote sensing technologies and machine learning come with many challenges. In this paper, we address the problem of data processing and experimental design to train machine learning models. Unmanned aerial vehicles (UAVs) captures vast amounts of data when flying over experimental fields. Managing, cleaning and processing this data into a coherent dataset to train a statistical model requires significant effort. Although there is no unique solution to this vast and multi-disciplinary issue, the hurdle can be cleared with sophisticated experimental design and a solid framework of multiple targeted solutions. In what follows, we cover our setup to solve this complex task.

This research paper aims to explain rigorously the process of transitioning from raw UAV acquisitions to a time series of multispectral images acquired throughout the life cycle of winter wheat. We begin by introducing the sensor type, the field set-up and image acquisition modalities, followed by orthomosaic generation and dataset preparation, concluding with potential avenues for further research.

Material and methods

Field trial

The field trial 2023 in Obersiebenbrunn was managed by Saatzucht Edelhof and comprised 1064 plots of winter wheat with 256 different genotypes grown in two replications and two treatments, with and without irrigation (Fig. 1). In this setup, 7 ground control points (GCPs) were deployed on the fields. Six of them were used for geolocation and the remaining one was kept for validation purposes during the initial inspections. GCPs are con-

The setup on-site

	replication 1	replication 2	
no water	genotype 1	genotype 1	
water	genotype 1	genotype 1	
no water	genotype 2	genotype 2	
water	genotype 2	genotype 2	
no water	genotype 3	genotype 3	
water	genotype 3	genotype 3	
no water	genotype 4	genotype 4	

10m
1.5m

Ground Control Point from meter to sub-pixel location accuracy

1064 total plots

2 replications of each plot for better statistical robustness

plots irrigated and not to assess drought resistance

250+ genotypes to test at the same time

… and genotypes of different breeding generations

Figure 1 Scheme of the field trial at Obersiebenbrunn with two replications and two treatments per 256 different genotypes. Note that in practice, replications with the same cultivar are randomly distributed to account for spatial variation.

crete plaques with a checkerboard pattern deployed directly on the field (Fig. 2). The centre of the GCP is measured with a highly accurate global navigation satellite system (GNSS). In this research, we utilize a Ublox F9P GNSS in conjunction with a calibrated survey GNSS multiband antenna. The absolute accuracy fluctuates but consistently remains below a centimetre. In each image where a GCP is visible, the central pixel can then be manually assigned by an operator to the real world coordinates acquired with the high-accuracy GNSS. By performing this procedure on multiple images (usually around 20), it is possible to triangulate the position of the remaining pixels from the ground truth ones of the CGPs. The result of this operation is a greatly reduced geolocation error in comparison to regular direct sensor orientation through GNSS.

Drone acquisition

In our experiment, we used a custom Hexacopter, which was based on a modified Tarot 690 Proframe and equipped with a Cube Orange flight controller running Ardupilot8. This setup was chosen for its stability, manoeuvrability and ability to carry payloads of more than 500 g. We employ an Altum-PT multispectral camera (https://ageagle.com/drone-sensors/altum-pt-camera/) which captures signals across eight different spectral bands including panchromatic, RGB, red-edge, near infra-red (NIR), and long wave infra-red (LWIR). Table 1 presents an overview of the acquired spectral bands. These spectral bands are essential for computing important indices such as the normalised difference vegetation index (NDVI), which provides valuable insights into plant health and productivity. The formula used for the calculation of the NDVI is as follows: NDVI = (NIR-R)/(NIR+R). Here, NIR is the near infrared band, R is the red band and NDVI is the index value for a pixel. It is worth mentioning that other indices can be computed. The choice depends on the phenotypic trait under investigation and the specific cultivar under analysis (Chang-Brahim 2024). Before each flight, the camera is calibrated using a standardised target, *e.g.* a calibration reflectance panel with pre-measured reflectance values across the visible and near-infrared spectrum (Fig. 3). This calibration step is crucial to correct for environmental variations, such as fluctuations in luminosity caused by clouds or other environmental factors. We choose a flight altitude of 60 m, resulting in a spatial resolution of 2.5 cm per pixel. This altitude was selected to balance the need for high-

Figure 2 Example of a GCP in the field of Obersiebenbrunn during the 2023 measurement campaign. The image is presented in the panchromatic channel, and it is acquired at a height of 60 m.

Figure 3 Calibration plaque with known reflectance. This image is captured before each flight and is instrumental in obtaining physically accurate spectral measurements.

Table 1 Description of the different bands acquired by the Altum Camera in the 2023 measurement campaign.

Channel	Wavelength	Usage in precision agriculture
Red (R)	654-682 nm	They are primarily used for monitoring plant health. It helps in identifying vegeta-
Green (G)	533-587 nm	Essential for assessing chlorophyll content in plants. High chlorophyll content
Blue (B)	453-508 nm	Useful for analysing water content in vegetation. This can help in irrigation plan-
Red edge (RE)	705-729 nm	This band is sensitive to changes in chlorophyll content and can detect subtle
Near infrared (NIR)	785 - 899 nm	This band is used for assessing the overall health of the plant. It is also used for
Long-wave infrared (LWIR)	8000-14000 nm	This band measures temperature variations in crops and soil, which can be indica-tive of plant health and water needs.

resolution data with manageable data volumes. Although lower flight altitudes such as 15 or 30 m are possible, we opt for 60 m to decrease the acquisition cost and required resources. Additionally, our drone is equipped with a GNSS tracker to register its position and geolocalise each pixel acquired during the flights. This is combined with GCPs to ensure precise spatial referencing of the collected data, allowing for a precise reconstruction of the orthomosaic.

Orthomosaic generation
Images captured by drones undergo extensive processing to create a standardised dataset. This involves calibration, stitching, geolocation and orthorectification, culminating in the generation of an orthomosaic. Various commercial photogrammetry programmes offer solutions for these procedures. For our study, Pix4Dmapper was used, a comprehensive software facilitating the creation of orthomosaics from geolocated images (Yang *et al.,* 2020).

The procedure employed is the following:
Image Orientation and Camera Calibration: Initial steps involve correcting lens distortions and aligning images geometrically based on camera specifics.
Image Matching: Overlapping images are merged to create a seamless composite, aligning features and removing seams.
Geolocation: Spatial coordinates are assigned to each pixel, using GCPs for enhanced precision, though their application in wheat studies and automated phenotyping remains limited.
Orthorectification: Adjusts for terrain distortions using a Digital Surface Model (DSM).
Mosaicing: Orthorectified images are combined into a cohesive orthomosaic, blending pixel values for a consistent representation.
Radiometric Calibration: Standardises pixel values by correcting for sensor differences and atmospheric conditions, utilising reference targets (see Fig. 3) to achieve accurate reflectance maps.

Analysis by Pix4Dmapper resulted in eight orthomosaics, one for each channel andfor every drone flight. For a practical procedure, please refer to the Supplementary material (Appendix).

Dataset creation
It's important to organise the dataset into plots for machine learning purposes, as they serve as the fundamental unit of reference

for expert-assessed measurements in the field. The positions of the four extremal points for each plot using GNSS technology are measured, resulting in 1064 tuples. These tuples are then converted into a shape file containing 1064 masks. The subsequent step involves applying an intersection operation to select only the reflectance values of the pixels associated with an experimental plot (Fig. 4).

This approach offers several advantages, avoiding for instance the use of heuristics to discern between relevant and irrelevant pixels (*e.g.* see the NDVI selection applied by Zhang *et al.*, 2019). However, this operation does not yield a clear rectangular image, but a generic quadrilateral embedded in pixels with no value. We opt to apply a homography and then a perspective transformation to map all points from the parallelogram to the rectangle with the
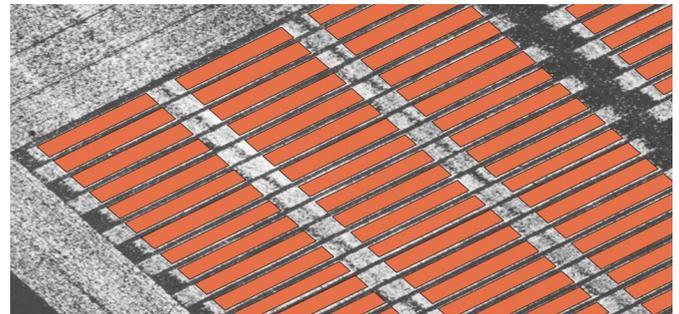
Figure 4 Detail of the representation of the plot masks (in orange) juxtaposed on the NDVI orthomosaic.

same shape. Consequently, the dataset is more standardised and is ready to use for any machine learning practitioner. To achieve this result, a resampling algorithm is used to generate the pixel values in the resultant image. In essence, this algorithm uses a square context window to replace the target pixel's value in the reconstructed image with the weighted average of neighbouring pixels from the original image. This averaging process may incorporate various weights based on the kernel function employed. In this particular methodology, we conducted a comparative analysis among four widely used resampling functions. Among them, the Lanczos resampling kernel (Fig. 7) is renowned for being the most performant (Turkowski 1990) since it constitutes a close approximation of the theoretically optimal sinc function. Nevertheless, we performed a benchmark study to assess the marginal gain by using three different kernels (nearest neighbour, bilinear and bicubic), to choose which one to select for the data processing pipeline.

The approach is done both considering the mean square errors (MSEs) between the various algorithms and their runtimes (Fig. 8).

This preprocessing procedure is repeated for each set of images acquired on every UAV flight conducted. However, due to particularly rainy weather in April and May 2023 and a drone malfunction during a flight in June, which necessitated repairs, the flights faced challenges. These complications highlight the main drawbacks of UAV technology: dependence on weather conditions and the risk of drone malfunction. Despite these challenges, seven full flights and two partial flights were completed. The flight dates (*i.e.* March 22, April 18 & 27, May 15 & 24, June 5 & 14) were initially scheduled every two weeks to ensure temporally close spectral readings of the plots, facilitating the monitoring of the physiological traits of the plants, which depend on various factors, including climate, weather, and genotype.

For storage, the orthomosaics for each plot are concatenated along the time dimension. This results in 1064 tensors, one for each plot, with four dimensions. The first dimension represents time, the second denotes spectral channels, and the remaining two dimensions represent image width and height (Fig. 5). To annotate the dataset, Staatzucht Edelhof supplies precise expert-assessed scores and phenotypic measurements for each plot, including yellow rust disease scores, water stress scores and plant height.

Results and discussion

In this section, we present the metrics showing the improvement achieved by GCPs and the results of the benchmark performed for the different kernels. Fig. 6 depicts the geolocation bias between the images originally acquired and the coordinates computed

using GCPs. The results are also reported in Supplementary Table S1, including the root mean squared errors (RMSEs) for the geolocation errors.

The first important consideration is that the GCPs contribute greatly to the correct geolocation of the orthomosaics' pixels. The experimental plots in Obersiebenbrunn have a width of 1.5 m, therefore a geolocation error of 2 m would incorrectly assign one pixel to another plot, introducing a bias in the measure and reducing the overall data quality. After the first flight, to assess the accuracy of our geolocation process utilising GCPs, we use a single control point as check point to evaluate the robustness of our system. The geolocation errors are 0.0053 m in X, -0.0271 m in Y, and -0.0854 m in Z. The projection error is determined to be 0.6876 pixels, with all measurements verified and marked. These results highlight the reliability and effectiveness of our geolocation methodology.

Regarding the choice of the resampling algorithm one resampled element in the NDVI channel reconstructed with different kernels is presented in Fig. 7. Fig. 8 shows a benchmark comparing the resampling algorithms. We compared the resampled images with one obtained using the Lanczos kernel. These measures are averaged across the reconstruction details of 300 distinct images for each channel, summing up to 2100 reconstructions. We plot the mean squared errors (MSEs) normalised by band variance, which enables a direct band comparison.

The benchmarks of the runtimes of the 4 kernels are presented on the same image data. Based on the benchmark results, we favour bicubic resampling. While Lanczos resampling may offer marginal improvements in reconstruction quality, the double increase in computational runtime outweighs the benefits, especially considering future scalability within a larger framework (Fig. 9).

As a conclusion, we have proposed an efficient and exhaustive operational method for capturing multispectral and multi-temporal data for machine learning purposes. Our method accounts for the combined requirements and difficulties of the fields of agriculture, plant breeding, remote sensing, and machine learning. We have used it to compile a ready-to-use dataset for machine learning purposes. To our knowledge, this work is the first to propose and implement a systematic pipeline of such high
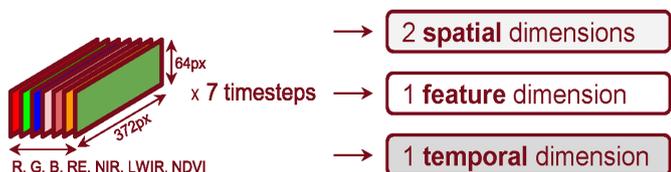
Figure 5 One sample in the dataset. The number of timesteps depends on the number of flights performed during the measurement campaign.
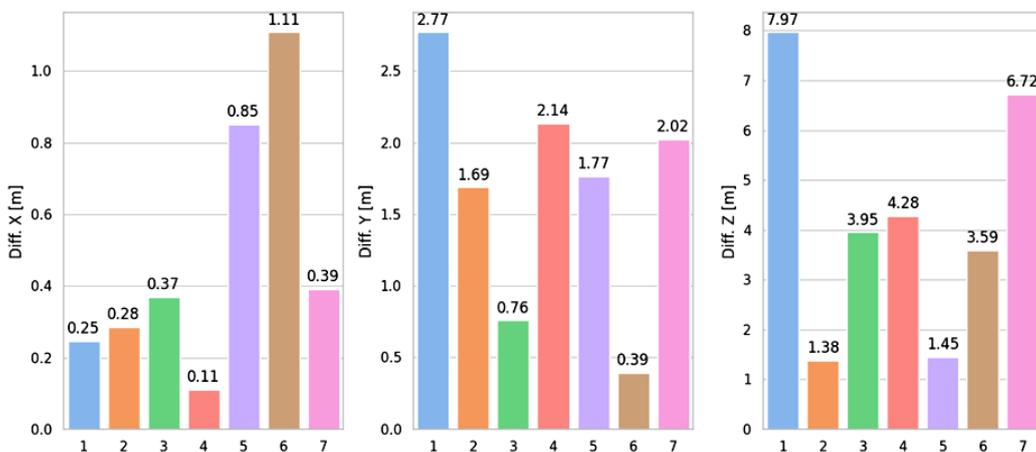
Figure 6 Geolocation error per coordinates: bias between the position acquired by the UAV and the position obtained from the GCPs. The numbers on the x-axis correspond to the flight dates.
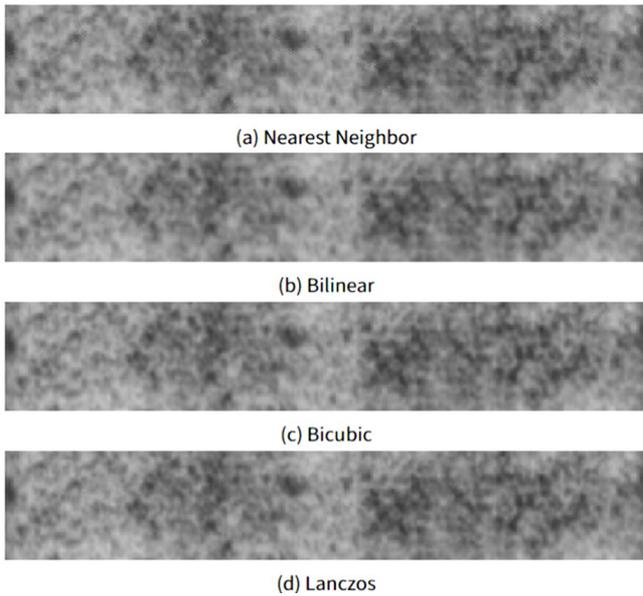
Figure 7 Example of a single reconstructed plot using the four different kernels. Artefacts are visible in the middle and right parts of (a) and (b) but absent in (c) and (d). The bandwidth presented is panchromatic.
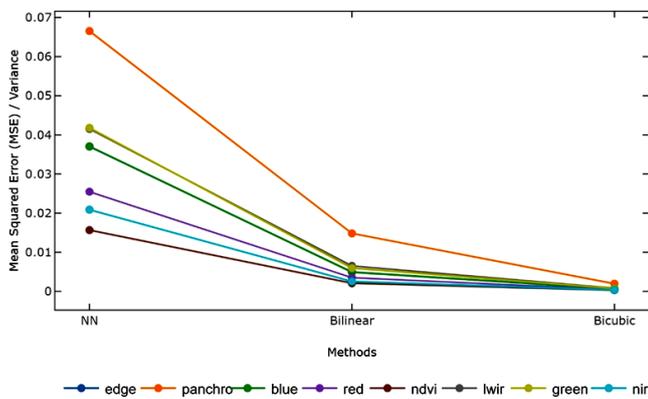
Figure 8 Comparison of images resampled with different methods with the Lanczos resampled image. Mean Squared Errors are normalised to account for variance, acknowledging the wavelength difference in orders of magnitude between RGB and the LWIR band.
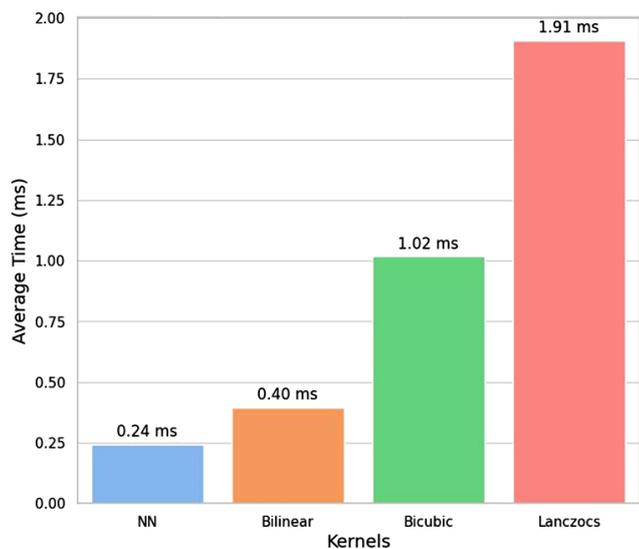
Figure 9 Average runtimes for different resampling algorithms. Results represent an average of over 300 runs, with each run reconstructing a single image.

quality for multi-temporal acquisition in plant breeding paired with such comprehensive ground-truth annotations.

We have devised a procedure to compile datasets tailored for machine learning applications, particularly for training deep learning models. It is meant to address both time and space complexities relevant to remote phenotyping and precision agriculture applications. Other operational concerns are left unaddressed, such as the deployment and automation of the pipeline to scalable cloud platforms. In addition to this, several steps still involve manual work that could be automated, for example manually creating plot bounding boxes.

Acknowledgements

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Supplementary material

Appendix: Pix4Dmapper operative procedure
(i) Open Pix4D and create a new project.
(ii) Load the images into the project, ensuring they are organised in the same folder structure as acquired by the camera. For our project, we used an Altum-PT camera, which divides single images into spectral bands.
(iii) In the new project window, navigate to *Advanced* settings and set the altitude of the input images to 0.
(iv) Select ETRS (European Terrestrial Reference System) as the output coordinate system.
(v) In the processing options, deselect *point cloud* and *mesh,* leaving only the initial processing enabled to reduce hardware load.
(vi) Proceed to the DSM (Digital Surface Model) tab and choose GeoTiff as the output format. Opt *Merge Tiles* since the orthomosaic is unnecessary for this project.
(vii) Select the desired indices, including the 7 spectral bands and precomputed NDVI, for standardised output.
(viii) Ensure the index calculator selects the area over the calibration tile. Click *Calibration*, excluding LWIR parameter calibration as the thermal camera is precalibrated.
(ix) Click *Merge Tiles* in the reflectance map section.
(x) Review the automatically generated quality report to verify the coverage and flatness of the point cloud.

(xi) Access the *Manage GCPs* panel, change the GCPs coordinate system to WGS84 (World Geodetic System 1984), and update the Geoid height as per acquisition system specifications. In our case, the height difference between WGS84 ellipsoid and geoid.
(xii) Import GCPs.
(xiii) Measure GCPs in the image by zooming out to locate the checkerboard pattern and clicking precisely in the middle of the chess pattern between 15 to 20 images, ensuring a reconstructed mean error of less than a single pixel.
(xiv) After measuring GCPs, navigate to *Process* and select *Reoptimize*.
(xv) Deselect initial processing and choose the 2nd and 3rd processes. Click *Start* to initiate processing with the selected options.

The procedure guidelines outline the tailored steps for generating orthomosaics specifically designed for operations in Obersiebenbrunn. It is important to note that for generalization to other cases, parameters and settings may require modification according to the specific context and the desired coordinate systems. Adjustments to parameters and settings should be made to accommodate different environments, equipment, and project requirements.

Table S1 Geolocation error between the position acquired by the UAV and the position corrected with the GCPs. Mean error and standard error (SEM) represent the mean of the geolocation errors and their variance. RMSE represents the Root Mean Square Error between the geolocation of the UAV and the one corrected using the GCPs. All measures are in m.

	X (t = 1)	Y (t = 1)	Z (t = 1)	X (t = 2)	Y (t = 2)	Z (t = 2)
Mean Error	0.22	3.04	1.43	0.45	1.08	4.04
SEM	0.53	1.13	1.71	0.47	0.57	0.45
RMSE	0.57	3.25	2.23	0.65	1.23	4.07
	X (t = 3)	Y (t = 3)	Z (t = 3)	X (t = 4)	Y (t = 4)	Z (t = 4)
Mean Error	0.69	2.51	3.55	0.36	0.77	3.94
SEM	0.62	1.05	1.33	0.43	0.52	0.39
RMSE	0.93	2.72	3.8	0.56	0.93	3.96
	X (t = 5)	Y (t = 5)	Z (t = 5)	X (t = 6)	Y (t = 6)	Z (t = 6)
Mean Error	0.81	1.68	1.13	0.07	2.25	4.73
SEM	0.58	0.67	1.57	0.61	1.27	1.77
RMSE	1	1.8	1.94	0.61	2.59	5.05
	X (t = 7)	Y (t = 7)	Z (t = 7)			
Mean Error	-1.23	-0.48	3.63			
SEM	1.6	1.2	1.11			
RMSE	2.03	1.29	3.8			

WheatVIZ - An interdisciplinary project to accelerate the breeding of drought-tolerant winter wheat via cutting-edge genomics and UAV-based phenotyping by integrating explainable AI

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Abstract

Water scarcity is considered a key threat for the 21st century: by 2030 the projected global annual water demand will be 40% above available supplies (Gilbert, 2010). In addition, even climate projections considering the Shared Socioeconomic Pathway 1 (SSP1) narrative predict a decrease in precipitation of around 700 mm and a change in soil water content of up to -62 kg m^{-2} for parts of Europe for the year 2060 relative to 2020. Together with the additionally projected higher temperatures, the frequency of crop water shortage periods will likely increase drastically (Qin *et al.*, 2023), pointing to high risk for crop production in future, also affecting wheat, one of the key staple crops worldwide and particularly of Western Europe. Simulation model predictions point to a high risk for wheat production under the foreseen climatic conditions with hot temperatures and drought periods, especially when occurring at a sensitive developmental stage, such as germination, tillering, flowering, or grain filling (Senapati *et al.*, 2021). All these factors make it imperative to accelerate the breeding process towards more drought (and heat) tolerant varieties to cope with the speed and magnitude of climate change, especially on a local scale. Therefore, we have initiated the WheatVIZ project, an interdisciplinary project including two centers of the AIT Austrian Institute of Technology, the Center for Health & Bioresources and the Center for Vision, Automation & Control, and the breeding company Saatzucht Edelhof GmbH together with the Agricultural Technical School Edelhof (LFS Edelhof). The WheatVIZ project aims at accelerating several steps of the winter wheat breeding process by using state-of-the-art airborne phenotyping and genotyping technologies supported by explainable artificial intelligence (xAI) methods (Figure 1).

In the initial phase of the project, we will gather multi-year historical yield data, encompassing more than 100 phenotypic parameters (such as disease scores, gluten and protein content, etc.) from selected wheat lines originating from both dry and wet regions. Subsequently, this data will be compiled, digitized, curated, and thoroughly analyzed to explore correlations with local weather conditions. This analysis will specifically consider the factors solar radiation, minimum and maximum temperatures, and sum of precipitation on a daily basis. The second and more resource-intensive stage of the project involves conducting a field

trial in Eastern Austria, which is characterized by a typically hot and dry climate. Approximately 250 winter wheat lines from three distinct breeding generations (W1-3) will be cultivated under two contrasting soil moisture conditions: irrigated and non-irrigated. The non-irrigated condition is designed to expose the crops to naturally occurring drought events during wheat development.

Throughout the growth stages, various agronomic, morphological, and physiological traits will be examined through traditional methods involving visual assessments and laboratory analyses, as typically employed by breeders. However, recognizing the time-consuming and labor-intensive nature of these conventional approaches, a primary objective of the project is to establish a high-throughput remote phenotyping process. This will be facilitated by unmanned aerial vehicles (UAVs) and machine learning (ML). To achieve this, multiple flights will be scheduled throughout the entire growing season of winter wheat, covering different developmental stages. The raw data from the mounted multispectral camera will not only allow the extrapolation of plant height but also enable the computation of various spectral indices (such as the normalized difference vegetation index, NDVI) on a plot level over time. This approach aims to estimate the health status of each genotype, providing accurate and representative information on drought stress traits. To fully exploit the information-rich content obtained, specific ML models, capable of addressing the spatial complexity inherent in high-resolution images, such as convolutional neural networks (CNNs), will be customized to meet the requirements of the WheatVIZ project.

Another objective of WheatVIZ, aimed at optimizing the breeding process, is the identification of genetic markers for drought tolerance within the examined breeding population. To achieve this goal, we will integrate various phenotypic data (both traditionally and digitally collected) with genotypic data obtained through single-nucleotide polymorphism (SNP) genotyping, specifically using the 25k wheat array from SGS Institut Fresenius GmbH. This integration will be carried out through classical genome-wide association studies (GWAS) approaches. Despite the widespread use of GWAS in identifying marker-trait associations, it is important to note that these approaches still possess notable limitations (Tibbs Cortes *et al.*, 2021). Therefore, our aim is to contribute to the further development and optimization of machine
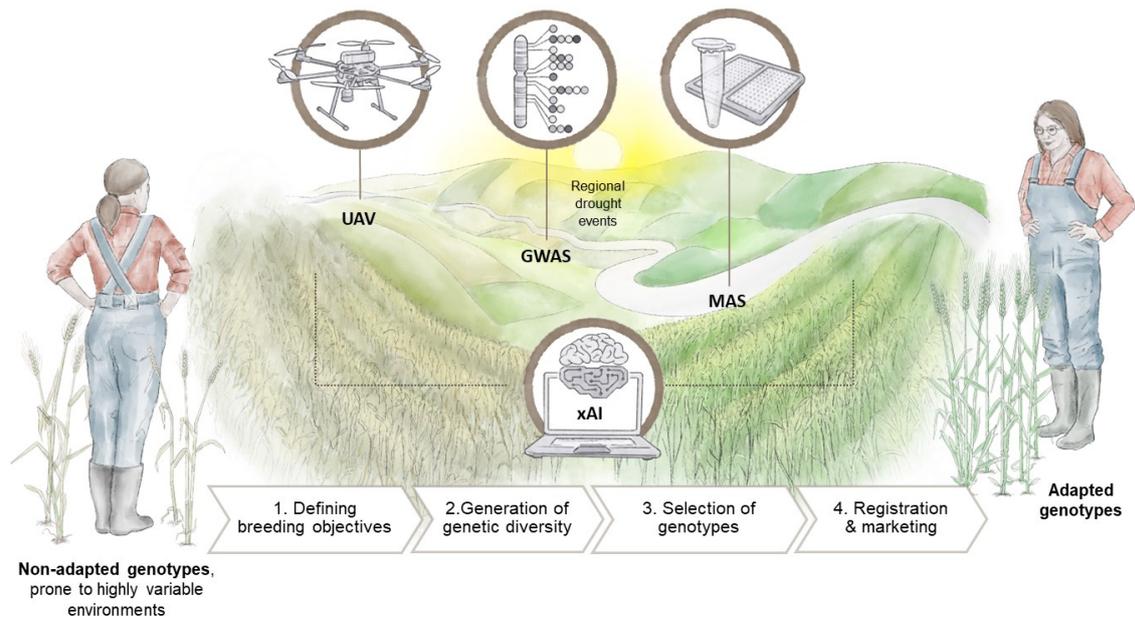
Figure 1 The road to drought-tolerant wheat genotypes remains tedious and time-consuming, but cutting-edge technologies such as UAV-based phenotyping, GWAS, and ultimately MAS promise to speed up the breeding process. These tools together with explainable AI as are going to be utilized in the WheatVIZ project to support decision-making within the entire breeding process, from the definition of breeding goals to the final step of registration and marketing (designed by Tatjana Hirschmugl; www.scillustration.at).

learning (ML)-based GWAS models. This includes considerations for aspects of explainability, as already explored by Mieth *et al*. (2021). The diverse array of tools at our disposal will be compared for their applicability on this real-life wheat dataset.

By leveraging these technological capabilities, breeders can significantly expedite the development of new varieties with enhanced traits, such as drought tolerance. This acceleration not only underscores the progress in science and technology but also holds the potential to address pivotal challenges in agriculture, including feeding a growing global population and mitigating the impacts of climate change on crop production. However, the realization of these benefits hinges on the integration of data and optimized processes into the hands of breeders. Consequently, WheatVIZ places a strong emphasis on bridging the gap between scientific achievements and practical application through a closely collaborative effort between breeders and scientists.

Keywords

Drought tolerance · machine learning · marker trait associations · remote phenotyping · wheat breeding

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Postprocessing and geopositioning of drone images to detect fusarium head blight

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Abstract

Fusarium head blight (FHB) severely threatens food security in global cereal production. To this end, resistance breeding requires the assessment of large numbers of breeding lines. These tasks are typically performed manually by the 'trained eye' of an experienced breeder. However, this procedure is laborious, expensive, time-consuming, and prone to human errors. This project aims to automatically capture high-resolution images of the field plots employing a drone (Matrice 300 RTK) equipped with a high-resolution RGB camera (PhaseOne 100MP, 150 mm lens) and then apply novel data analysis techniques that have not yet existed to detect, quantify and select the resistant breeding lines.

With our equipment, we captured the complete FHB experiment at eight time points during the FHB evaluation period in 2022. Fig. 1 sketches a usual flight. The 45° angled camera position, in combination with the flight height of 12 m above the ground, covers 6×4 m per image. From this image, we can determine the location of individual plots. From one individual plot, we can extract input pictures with the required resolution of ≈0.5 mm per pixel and the image quality needed for analysis with neural networks.

We analyzed all images and metadata to ensure the camera-drone system provided flawless and fast communication. Each image showed a highly nonlinear distortion and we developed a procedure to remove this distortion. We calculated the center of each image with the metadata and removed redundant images for efficiency.

Keywords

high-resolution imaging · neural networks · remote sensing · RGB · unmanned aerial vehicle

Acknowledgements
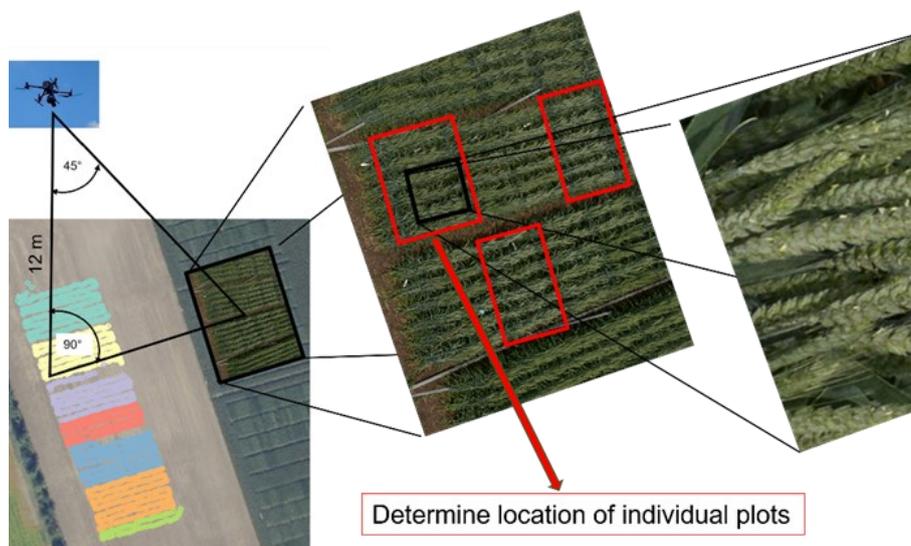
Figure 1 Flight with 45° angled camera position 12 m above the ground. We determine the location of individual plots. Then we extract input pictures with the required resolution of ≈0.5 mm per pixel and the quality needed for analysis with neural networks.

Further reading

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Introduction of a drone-based high throughput phenotyping system in cereal breeding at Martonvásár

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Abstract

In recent years, advances in Unmanned Aerial Vehicle (UAV) or drone technology and special sensors have made it possible to collect large amounts of reliable information on the plants we observe (Liu *et al.*, 2023). Long-range spectroscopic measurements are a tremendous help in surveying large areas in minutes, allowing coordinate-locational data to be recorded in seconds. With high-throughput phenotyping, experiments involving tens of thousands of plots can be easily traced, and by analysing the vast amount of data collected in a season, species-specific plant models from germination to maturity can be developed. Our aim is to process, understand and make practical use of the large data sets available on vegetation, one of the new challenges that arise with the digitalisation of crop production.

Drone flights have been carried out at the Agricultural Institute, Martonvásár, Hungary, since 2019. In addition, in autumn 2021 and 2022, an expanded reference variety trial was established, consisting of 11 winter wheat, barley, durum wheat and triticale genotypes, which are susceptible and/or tolerant to disease, drought and heat stress. The trial was artificially inoculated with leaf rust and additionally with *Pyrenophora teres* f. *maculata* in case of barley. Four out of eight randomly arranged replications were treated with fungicide. In each growing season the drone flew from spring onwards weekly over the plots at a height of 100 meters. Using the flight data and the flight plan, an automatically repeatable programme could be created to take weekly photos of the experimental area at the same coordinates, with centimetre accuracy. The drone forwarded the recorded data to the AGRON-Maps platform (AGRON Analytics Kft., Budaörs), where they were processed. Different vegetation indices were calculated from this database. Altogether, 17 phenotypic parameters were recorded, 47 spectral data were measured by the drone on 12 occasions, and all plots were harvested at maturity.

By analyzing the maps of the area flown over (orthomosaic and reflectance maps), it is possible to determine the spectral reflectance indices (Figure 1) calculated from the light absorbing and reflecting capacity of the leaf tissue at visible (λ=400-750 nm) and near-infrared (λ=750-1400 nm) wavelengths. With the vegetation indices, it is possible to show differences between the breeding lines in terms of disease resistance, insect infestation, drought tolerance, nutrient utilization capacity, plant tissue aging and photosynthetic activity.

The plants showed a special multispectral profile, and clear differences between the species were detected. It was also possible to differentiate between varieties, which can greatly help in the breeding processes in the near future. Graphical display of the principal component analysis data clearly reflected the plants' ripening process and therefore the separation of early and late maturing varieties. In experiments with fungicide treatments the pattern of the multispectral values of the varieties susceptible to leaf diseases could easily be distinguished from the resistant varieties. The heatmaps of these trials can also immediately be used in the selection process.

Keywords

Heatmaps · multispectral profiles · reflactance maps · UAV

Bányai J, Balassa G, Turbéki R, Bakonyi J, Cséplő M *et al.* (2024) Introduction of a drone-based high throughput phenotyping system in cereal breeding at Martonvásár. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 17-18. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5
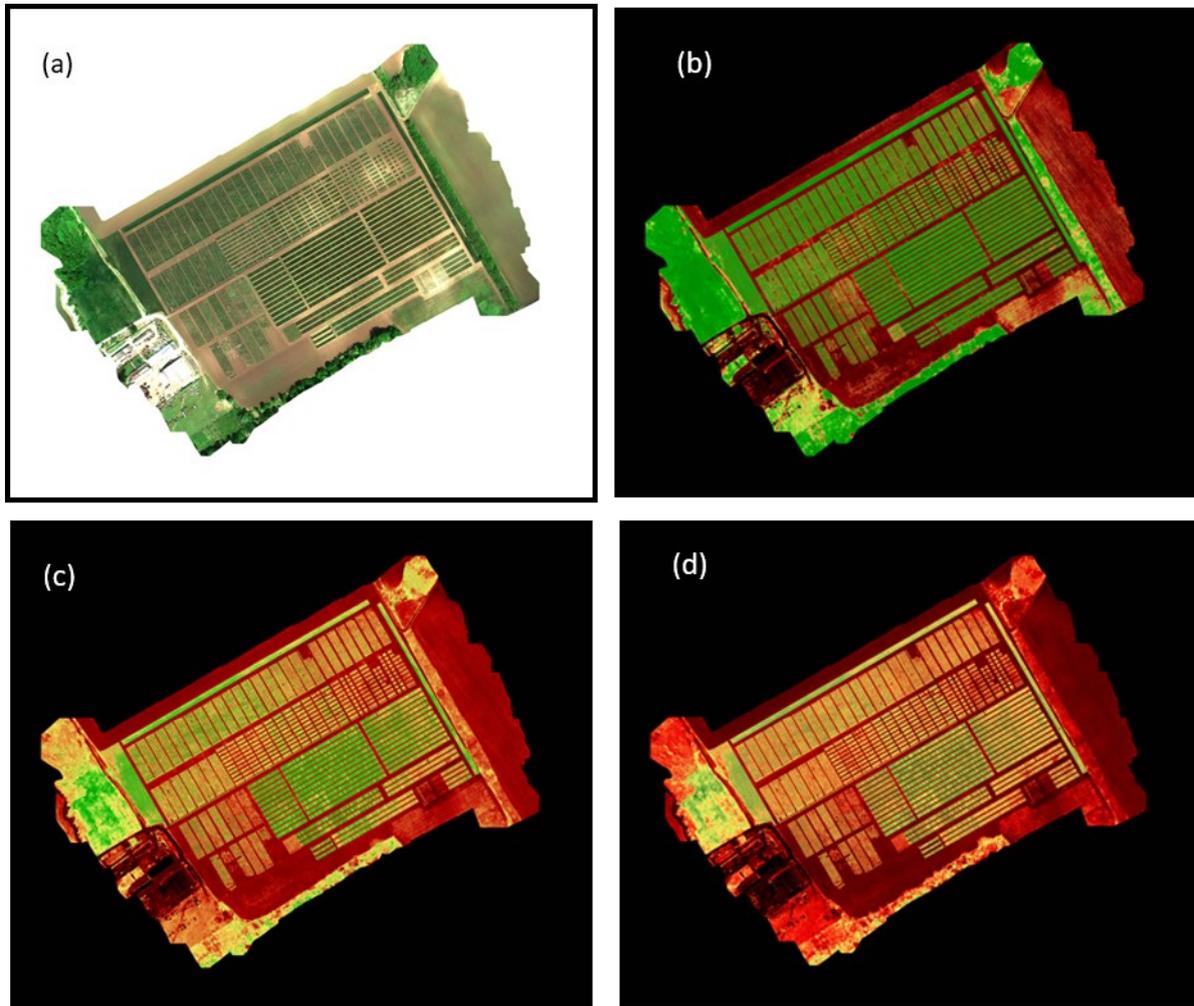
Figure 1 Maps of the experimental breeding field in Martonvásár, Hungary, created by different spectral reflectance indices: **a** RGB (Red, green and blue bands), **b** NDVI (Normalized Difference Vegetation Index), **c** GNDVI (Green Normalized Difference Vegetation Index) and **d** NDRE (Normalized Difference Red Edge) index.

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Phenotyping of winter wheat genotypes using UAV multispectral imaging

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Abstract

Weed competitiveness of 12 winter wheat cultivars ('Alixan', 'Bernstein', 'CCB Ingenio', 'Gorolka', 'Illico', 'Izalco CS', 'Marinka', 'Primorka', 'Reska', 'Savinja', 'Tata mata', 'Vulkan') was evaluated in three growing seasons (2020-2022) at the experimental station in Jablje, Slovenia. The selected cultivars exhibited substantial variation in their morphological, phenological, and agronomic traits. The experiment comprised three individual trials per growing season, each representing a distinct competitive scenario: (i) competition with common weed species; (ii) competition with barley (cv. 'Sandra') as a weed mimic; and (iii) a weed-free control. Conventional soil tillage involved autumn plowing to a depth of 25 cm, followed by seedbed preparation. Crop rotation in the fields included maize, winter wheat, winter barley and alfalfa, with winter wheat consistently following maize. Sowing density for winter wheat was 400 viable seeds/m². In the weed competitor trial, winter barley seeds were added to the wheat seeds at a density of 60 viable seeds/m² to simulate weed presence. The trials followed a randomized complete block design with four replications (1.25×6 m plot size). Seeding took place with a plot seeder (Wintersteiger Seedmech, Austria) between 10th and 25th October, depending on weather conditions. Weed control in trials 2 and 3 involved post-emergence herbicide application at growth stages BBCH 35-40. All plots received synthetic N fertilizers at a rate of 170 kg N ha⁻¹ in each growing season. Additionally, two applications of fungicides were applied at BBCH 37-39 and BBCH 59-65. Insecticides were applied if necessary.
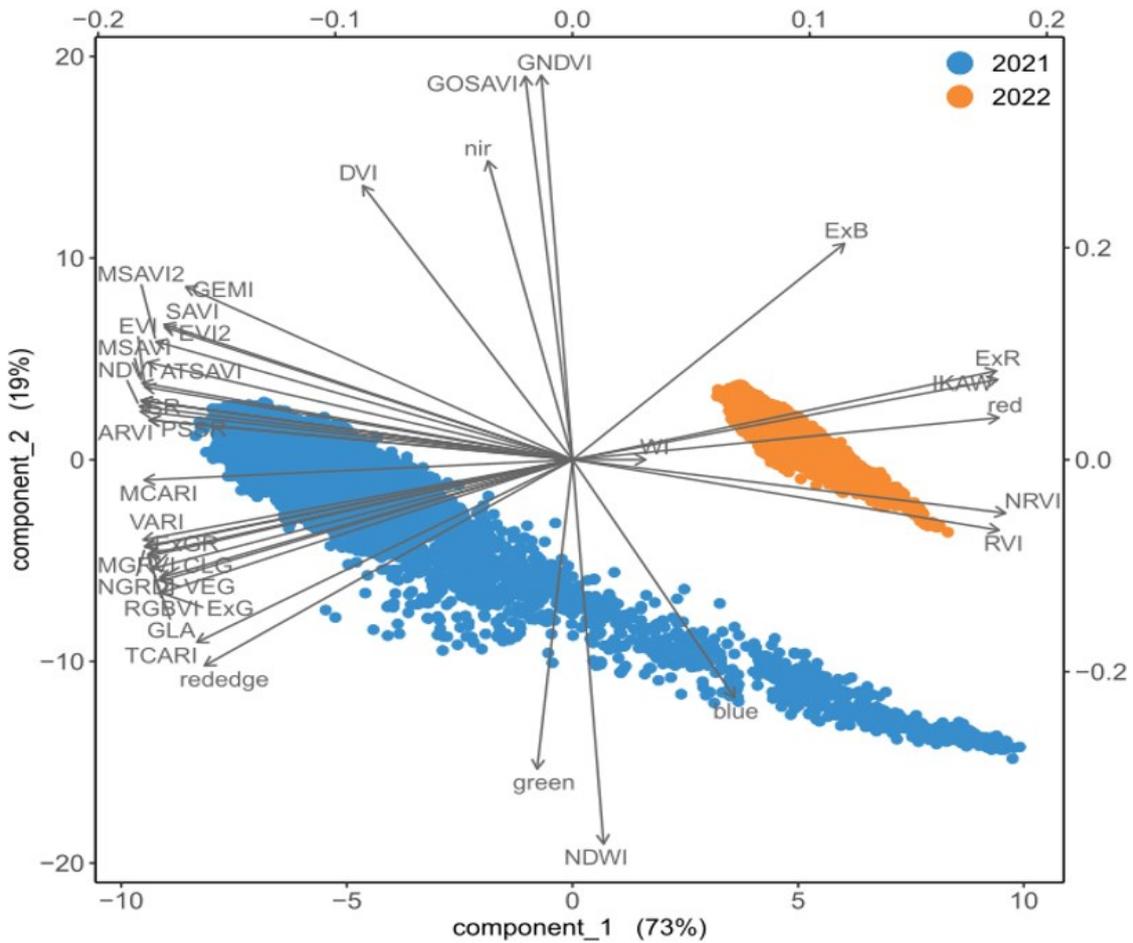
Aboveground biomass was systematically sampled at two growth stages, BBCH 60-62 and BBCH 75-85. A 30×30 cm square was randomly placed within each plot. Within this area, all plants were cut off at soil surface and fresh and dry (48 h, 80°C) weights were determined of the wheat, the weed mimics and weed plants. Harvesting of plots was done with a Quantum plot harvester (Wintersteiger). A grain sample of 2 kg underwent analysis by a Infratec Nova NIR analyzer (Foss, Denmark). Samples of the weed mimic trial underwent optical and manual sorting into wheat and barley grains. Barley and wheat grain masses were quantified and the percentage of barley grain mass was calculated.

Multispectral imaging was performed the days of biomass sampling, in 2021 and 2022, around midday. Images were acquired using a 5-band multispectral camera (MicaSense RedEdge-MX, Micasense, Seattle, WA), equipped with a down-welling light sensors (DLS), and mounted on an unmanned aerial vehicle (UAV; SkyHero Spyder GEO Edition quadcopter). Ground control points (GCP) were localised using a high-precision GNSS/GPS station (Stonex S9i, Stonex Srl, IT), and used for georeferencing. Radiometric calibration, orthorectification, generation of orthomosaics, and transformation to reflectance values were performed using Pix4Dmapper (Pix4D, Prilly, CH), while georeferencing was done using QGIS. A polygon shapefile was generated, where each microplot was split into 8×8 subplots, and per-pixel reflectance values were extracted. In total 35 vegetation indices related to canopy cover and plant physiology, were calculated. Together with the 5 bands, 40 features for each pixel were generated. For classification, mean spectra were calculated for each subplot (*i.e.* 64 mean spectra for each microplot), and for regression analysis, mean spectra were calculated for each plot (*i.e.* each biological replicate).

Principal component analysis (PCA) was used for data exploration and visualisation. Sparse partial least squares (sPLS) was used for dimensionality reduction, feature generation, and regression analysis. sPLS models were tuned to determine the optimal number of latent variables by using 50-times repeated 10-fold cross-validation. The performance of each sPLS discriminant analysis (sPLS-DA) model was assessed by 10-times repeated 10-fold cross-validation and by comparing three distance measures (maximum distance, Mahalanobis distance, centroids distance). The combination of lowest error rate and lowest number of latent variables indicated the best result and was used for further analysis. Variable importance in projection analysis (VIP) was used for assessing the importance of individual features for regression and classification.

Regression analysis of fresh and dry biomass, and yield was conducted using sPLS. sPLS-DA dimensionality reduced data were used as features for support vector machine classification (sPLS-SVM). Classification analysis was performed for variety and treatment identification. Hyperparameter tuning was performed using a grid search, testing all possible combinations of sigma and the

Žibrat U, Vončina A, Knapič M, Kolmanič A, Grausgruber H, *et al.* (2024) Phenotyping of winter wheat genotypes using UAV multispectral imaging. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 19-22. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5

Figure 1 Biplot of the principal component analysis of vegetation indices data.

cost function ("c") along a pre-defined vector. Models were validated using 10-times repeated 10-fold cross-validation. The training set was used for algorithm training, and the test set evaluated the developed models on data the algorithm has not been exposed to. All results are presented on the test set.

PCA showed two distinct clusters, corresponding to years 2021 and 2022 (Fig. 1). This indicated, that spectral reflectance of plants from the two years was significantly different, with a 100% classification accuracy (sPLS-SVM). This is a consequence of two effects, *i.e.* differences in weather conditions - the 2021/2022 growing season had significanlty less rainfall and lower air humidity - and differences in growth substrate (the distance between the experimental fields was approximately 1.5 km). All further analyses were performed separately for each year.

Identification of all 12 varieties achieved an accuracy of 45%. Further analysis of sensitivity and specificity shows that 'Izalco' was classified with almost 100% accuracy, others ('Illico', 'Primorka') with accuracies above 85%; 'Savinja', 'Bernstein', 'Vulkan', and 'Tata Mata' had sensitivities below 20%, most of the data points were missclassified. On the other hand, specificities of these varieties were above 95%. Overall, the algorithm behaved conservatively, preffering to avoid false positives, but missing true positives. These results indicate that some varieties are spectrally indistinguishable. For further analysis new groups of varieties were formed, based on these results. 'Alixan', 'Marinka', and 'Bernstein' formed group X91112, 'Savinja', 'Tata Mata', and

'Reska' group X568, and 'Izalco' and 'Vulkan' group X37. This grouping increased classification accuracy to approximately 70% and 55%, for 2021 and 2022, respectively (Fig. 2a). The difference between results on the test and training sets indicate that overfit was not present, the results are therefore reliable even on new data. Further separating the data by treatment increased accuracies, up to 80% for grouped varieties in the control trial. Worst results (acc. ≈60%) was achieved in the weedy trial, whereas the weed mimic trials achieved ≈70% accuracy (Fig. 2b). This indicates that the presence of barley influenced reflectance properties of wheat to a lesser extent than weeds. From the original 40 features, 14 were identified as relevant for variety classification (Fig. 3). By removing the remaining, superfluous features, classification accuracies remained at approximately the same levels.

Identification of treatments achieved an accuracy of >75 % for both years. Accuracies were improved by including variety identity as a new feature. The algorithm again behaved conservatively, but not to such an extent as for the identification of 12 varieties. The presence or absence of weed and barley significantly affects spectral properties of subplots, indicating the need for detailed image segmentation and filtering, to remove weed and barley data from the images.

Data pooled from both years, produced a root mean squared error (RMSE) and mean absolute error (MAE) for yield of 1057.5 and 866.4, respectively. Similar to variety identification, the data had
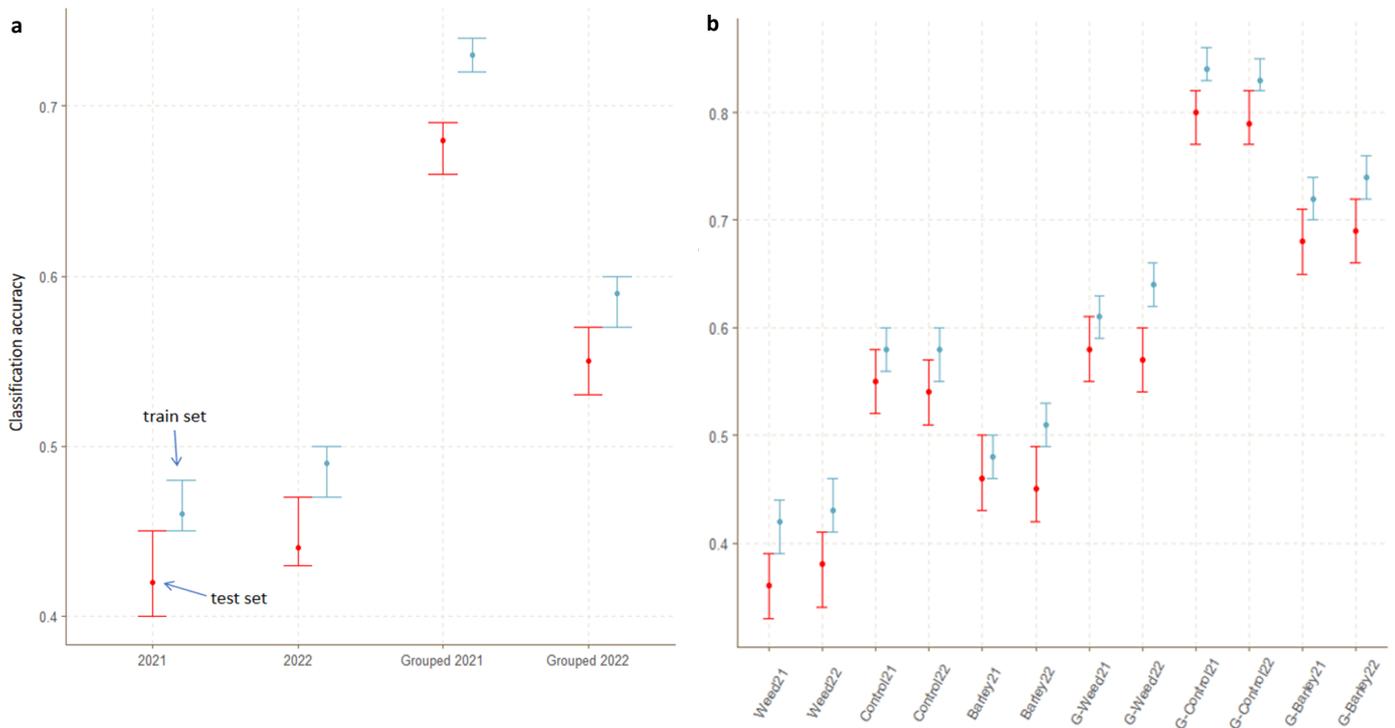
Figure 2 Comparison of variety classification results on the training and test set for individual varieties and grouped data: **a** separated by years; **b** separated by treatment and year (red bars: test set; blue bars: training set).
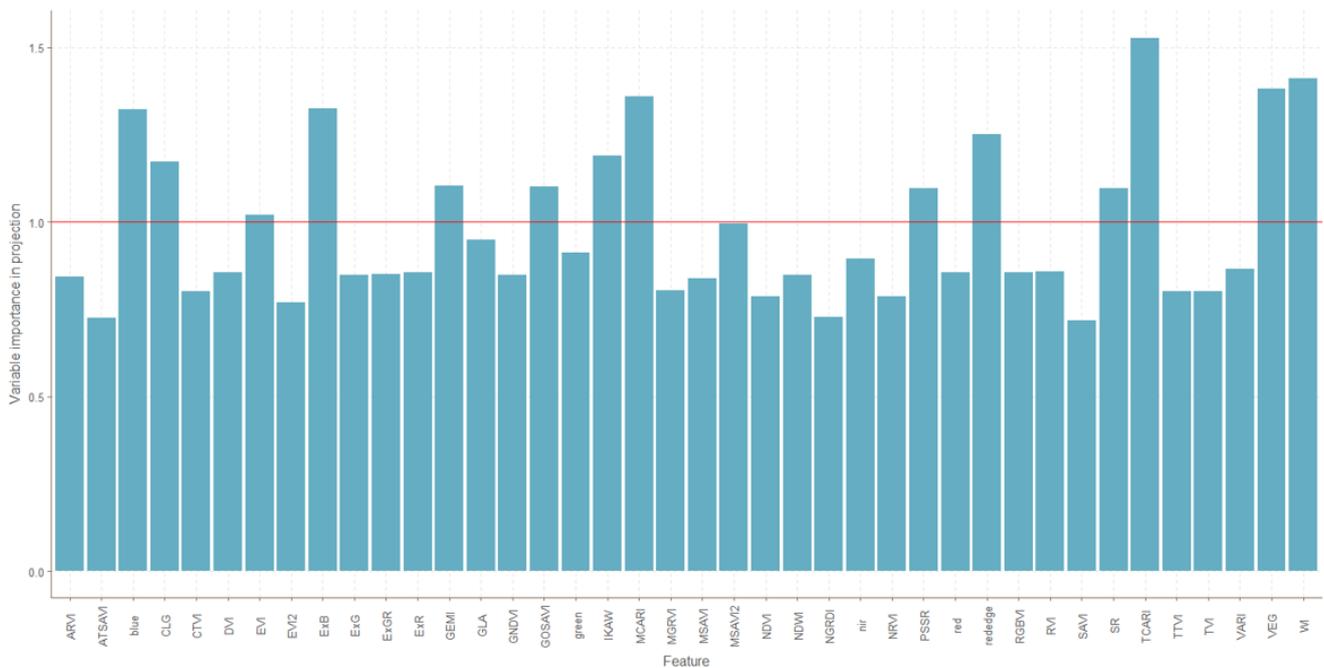
Figure 3 Variable importance in projection analysis for identification of wheat varieties.

to be analysed separately for each year, and results improved when data was split by treatment. Only prediction models on test data for weed and barley achieved acceptable RMSE and MAE, i.e. both of less than 10% of biomass value ranges. Results on data from 2022 were more accurate, both for biomass and yield. Yield prediction was also accurate on data, pooled from all three treatments in 2021. The errors or the differences between the observed values and the values predicted by the regression model follow a normal distribution, indicating that the models are unbiased.

The integration of multispectral imaging via drones demonstrates significant potential for enhancing high-throughput wheat phenotyping. While the application of sPLS regression and sPLS-SVM classification models holds promise, there is evident room for refinement. Notably, addressing the conservativeness in model outcomes calls for further research to optimize their predictive capabilities. Additionally, the conclusion underscores the necessity for augmenting the dataset to facilitate the development of more robust regression models. This comprehensive evaluation lays the groundwork for future advancements in precision agriculture,

emphasizing the importance of ongoing research and iterative improvements to unlock the full potential of these technologies in crop phenotyping.

Keywords

Acknowledgements

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Hyperspectral sensing for digital phenotyping in soybean breeding research

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Abstract

In soybean breeding, there is a need to observe specialty crop features such as biomass accumulation and ground cover at particular stages of development, biological di-nitrogen fixation, phenological adaptation to a given latitude, disease incidence, or plant water status. For such characters, a precise phenotyping is desirable for an in-depth characterization of germplasm and other breeding materials. High throughput phenotyping (HTP) using digital sensing tools such as cameras or other spectral sensors offers the rapid screening of these complex characters for large numbers of genotypes in field experiments. Apart from hand-held instruments and ground-based devices such as phenomobiles, unmanned aerial vehicles equipped with various cameras can be utilized in data collection for HTP (Araus et al., 2018). Drones carrying multi-band cameras have been used successfully to measure fresh biomass of soybean from images using different statistical prediction models (Ranđelović et al., 2023). Handheld hyperspectral devices have been utilized for collecting spectral information to calculate various spectral reflectance indices (SRI) describing different crop traits. In addition, an indirect measurement of nitrogen accumulation and the prediction of seed protein content in mature seed from spectral data collected at the flowering stage has been reported (Vollmann et al., 2022). For a successful integration of HTP into plant breeding, implementation research is necessary to confirm the reproducibility and meaningfulness of a phenotyping method using a given instrumental device on genetic materials of interest. In this research, a set of elite soybean genotypes was subject to hyperspectral measurement and calculation of SRI across multiple growing seasons in order to evaluate a hyperspectral phenotyping procedure for characterizing soybean germplasm.

A set of 140 elite soybean cultivars of either Chinese or European origin (for details see Yao et al., 2023) was grown at Tulln / Austria in five seasons from 2019 to 2023. A FieldSpec HandHeld 2 VNIR spectroradiometer (ASD Inc., Boulder, CO, USA) was used for collecting spectral reflectance data in the wavelength range from 325 to 1075 nm at the full flowering to early seed-filling stages of development. From spectral data, 43 different SRI were calculated to describe either biomass production including several other canopy parameters, nitrogen accumulation, or plant water status. Data collection procedures and the SRI used have been described in full detail by Vollmann et al. (2022). SRI were obtained from individual field plots, and different ANOVA models were used to evaluate the effects of factors such as growing season, replication, soybean genotype, origin of genotype (China vs. Europe), maturity group, and E-haplotype (i.e. E-gene composition at the 4 E-loci E1 to E4 determining the day-length sensitivity of time to flowering).

Implementation research on hyperspectral data collection from soybean field plots revealed the need for a large number of replicated measurements per plot in order to achieve a sufficiently high precision of SRI suitable for differentiating between genotypes. A data collection protocol utilizing 400 spectral replications per plot for SRI calculation was determined as useful for obtaining meaningful results. As an example for highly precise differentiation between genotypes, the water index WI_2 (Fig. 1) is presented for genotypes of different soybean stem termination type, i.e. indeterminate (dt2) or semi-determinate (Dt2) stem termination. In addition to appropriate timing of flowering, plant architecture mediated by the Dt2 locus is an important feature for adaptation and yield performance of soybeans under Central European growing conditions. However, the MADS-box transcription factor Dt2 has various side effects apart from modulating stem termination: The dominant allele Dt2 is causing semi-determinate stem growth, but also affects water use efficiency which might reduce drought stress tolerance of semi-determinate cultivars (Vollmann & Škrabišová, 2022). Therefore, HTP measurement of water status is of relevance. In the set of elite soybean cultivars investigated, almost all SRI were significantly affected by genotype, origin of cultivar, E-haplotype or maturity group classification. As an example, Chinese cultivars were considerably lower in carotenoid pigmentation than European cultivars. Heritability estimates were high for nitrogen-related SRI and lower for most of the water status SRI. Subsequently, the clustering of cultivars based on SRI data revealed associations which are obviously based on similarities in physiological properties (Fig. 2). Some of the clus-
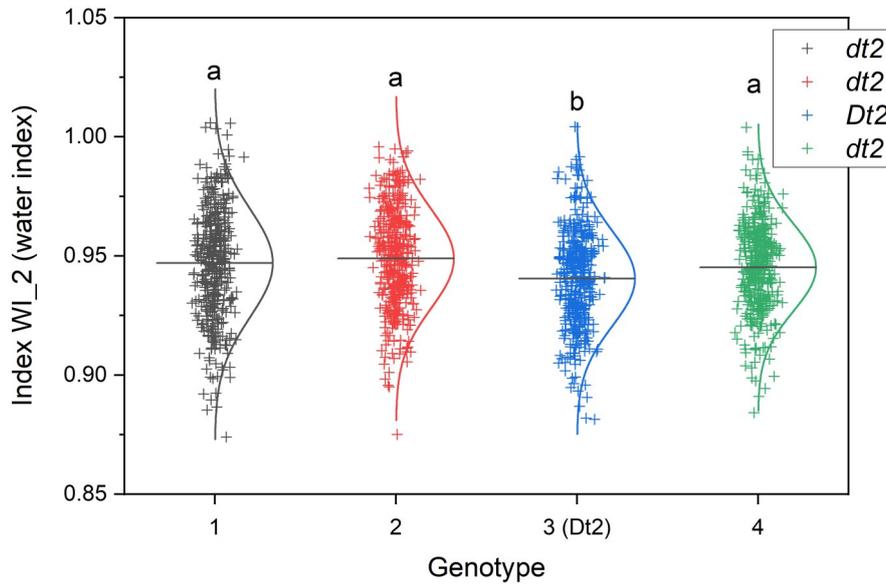
Figure 1 Spectral reflectance index WI_2 (water index) determined from 400 spectral replications per plot for indeterminate (*dt2*) or semi-determinate (*Dt2*) types of stem termination (letters above distributions: Tukey mean separation significant at $p < 0.0001$).
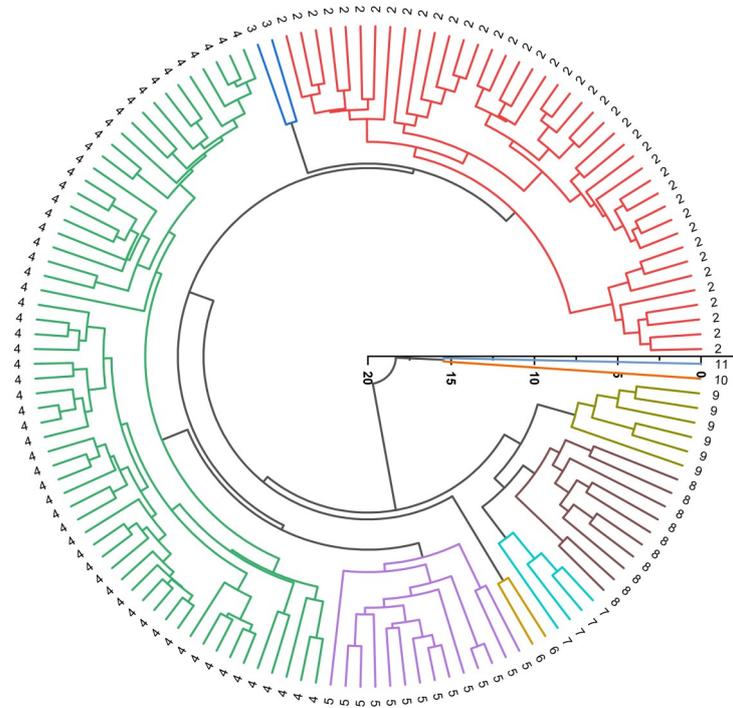
Figure 2 Dendrogram of 140 soybean cultivars based on association in 43 SRI (main clusters: "2" - early maturity European and Chinese cultivars, European sub-clusters; "4" - late maturity Chinese cluster with few European genotypes included; "5" - late maturity Italian and Serbian cultivars; "6" - Romania genotypes from Turda).

ters identified represent early maturity genotypes with separate Chinese or European sub-clusters, late maturity clusters with Serbian or Italian cultivars separated from Chinese ones, and late maturity Chinese genotypes.

In general, SRI calculated from hyperspectral reflectance data collected in field experiments are reproducible over growing seasons. They might be useful for a deeper description of cultivars, in gene characterization, for germplasm classification and in other fields of breeding research. Further implementation research is also required to optimize hyperspectral data collection from field experiments.

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Literal® and drone image processing for affordable phenotyping in plant breeding using Cloverfield

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Abstract

Hiphen stands as a pioneering force at the crossroads of innovation and agriculture, committed to shaping the future of plant breeding. With a rich decade-long journey marked by an unwavering pursuit of excellence, we specialize in plant measurements, utilizing cutting-edge sensing technologies in both field and controlled environments. Our core mission revolves around providing comprehensive insights into breeding programs and field trials. By seamlessly integrating Deep Learning models with profound agronomic knowledge, we have crafted solutions that empower informed decisions in plant breeding, ultimately reducing the time required to select market-demanded varieties.

As a trusted partner for agricultural companies globally, Hiphen has executed numerous projects, processed millions of plot images, and developed five innovative products (Fig. 1) to support various agronomic endeavors. At our core lies a commitment to efficiency and precision, recognizing the urgency inherent in the fast-paced realms of agriculture and plant breeding.

Our solutions are designed to help significantly reduce the time needed to select new plant varieties in breeding programs. By enhancing the quantity, repeatability, and quality of plant assessments, we collect valuable information that helps to streamline breeding decisions. This contribution accelerates agricultural innovation, ensuring the timely introduction of new and improved crop varieties. Key to our approach is the high level of accuracy achieved, with methodologies boasting strong correlations against visual scoring. This commitment to accuracy is more than a metric; it's a cornerstone of our philosophy, underscoring our dedication to providing reliable and trustworthy data to inform your breeding decisions.

Recognizing the uniqueness of each client's needs, Hiphen takes pride in offering tailor-made customer service. Our team collaborates closely with you, understanding specific goals and challenges, and providing personalized solutions that pave the way for success.
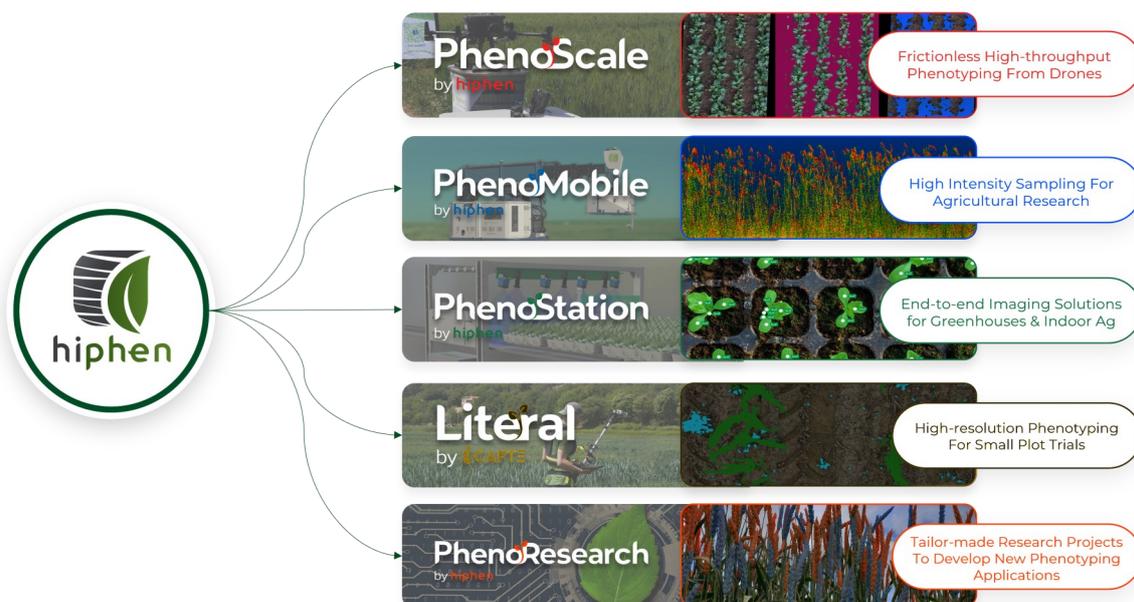
Figure 1 Product portfolio of Hiphen: From unmanned aerial and ground based vehicles to custom-made imaging systems (see also: https://www.hiphen-plant.com/phenotyping-devices/).

Bürgy A, Cheviet N, Comar A (2024) Literal® and drone image processing for affordable phenotyping in plant breeding using Cloverfield. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 27-28. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5

Navigating and understanding your data is effortless with Cloverfield (Fig. 2), our intuitive analytics interface. Designed for accessibility and user-friendliness, it not only gives you access to calculated traits in a comprehensible manner but also empowers you to derive meaningful insights from the wealth of information at your disposal like rating plot quality, correcting yield for mismanagement, determining trial success before harvest, predict yield or disease infestation and identify best performing lines from cross-trial analysis. This allows for more strategic planning, harvesting only the most valuable trials at a constant cost, and focusing on the most valuable genotypes that you wish to breed for.

Figure 2 Cloverfield - The all-in-one phenotyping data platform from data upload to crop analytics (see also: https://www.hiphen-plant.com/cloverfield/).

High-throughput phenotyping systems for controlled and natural environments

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Abstract

Photon Systems Instruments (PSI) stands as a beacon of technological prowess for over 25 years. Specializing in crafting sophisticated, top-tier instrumentation for biological sciences research, PSI has established itself as a global authority in the realm of advanced measurement and imaging of optical signals within plants and algae, particularly in the domain of chlorophyll fluorescence/reflectance techniques. At PSI, we have pioneered the development of chlorophyll fluorescence imaging systems, spearheading the digital revolution in image acquisition and processing. Our team comprises seasoned professionals, blending the expertise of plant scientists and engineers proficient in optics, electronics, software development, and automation. Our collective goal is to continually expand and innovate, furnishing the plant science community with cutting-edge scientific instruments to propel research endeavors forward. Our diverse product lines cater to a myriad of research needs, encompassing devices for chlorophyll fluorescence measurement and advanced imaging, state-of-the-art growth chambers and cultivation rooms for precise environmental control integrating innovative LED light sources and customized PlantScreen™ Phenotyping Systems operating in both plant-to-sensor and sensor-to-plant concept. High-throughput plant image-based phenotyping has emerged as a pivotal tool in both controlled environments and field conditions, revolutionizing the realm of plant research and breeding. This methodology enables the rapid and non-destructive assessment of diverse plant traits, ranging from growth dynamics to stress responses, at a scale previously unattainable. Leveraging our interdisciplinary expertise in plant science, robotics, engineering, and software development, we have unveiled the next generation of phenotyping solutions, the PlantScreen™ Systems (Fig. 1). These systems, designed for automated phenotyping in both controlled environments and field conditions, cater to a wide spectrum of plants, from *Arabidopsis* to mature crop plants. Applying advanced imaging technologies, coupled with robust data analysis algorithms, researchers can now efficiently collect and analyze vast amounts of plant data, facilitating accelerated breeding programs and precise agronomic decisions, bridging the gap between genotype and phenotype.

The key feature of PlantScreen™ high throughput image-based phenotyping systems is the integrative approach based on utilizing multiple imaging sensors to quantify various traits including

Figure 1 PlantScreen™ phenotyping Systems are integrated robotic solutions for high-precision digital plant phenotyping and plant cultivation throughout the entire life cycle, customized for both controlled and field environments.

Panzarová K, Abdelhakim LOA, Benedikty Z, Trtílek M (2024) High-throughput phenotyping systems for controlled and natural environments. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 29-30. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5

biomass, photosynthetic efficiency, canopy temperature, and leaf reflectance indices simultaneously. In addition precise control and synchronisation of cultivation and phenotyping protocols in PlantScreen Systems allow to simulate natural conditions and quantify the plant performance and productivity *in situ* in high throughput and high spatio-temporal resolution.

Keywords

Automated plant phenotyping · controlled cultivation · digital image analytics · integrative imaging methods · morpho-physiological · phenomics traits · robotics

Further reading

For a list of references see:
https://plantphenotyping.com/references/#publications
For ongoing and finished projects see:
https://psi.cz/about-company/projects/

Microbes for sustainable agriculture

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Abstract

Plant pathogens and pests cause up to 40% of yield loss each year worldwide (Savary *et al*., 2019; Venbrux *et al.*, 2023). Disaster events in crop and livestock production are responsible for an average loss of USD 123 billion per year or 5% of annual gross domestic product (FAO, 2023). The global trend for crop protection aims to reduce dependence on conventional pesticides in order to protect the environment. In 2020, the European Commission's Farm to Fork Strategy introduced two pesticide reduction targets with a 50% reduction in the use and risk of chemical pesticides and a 50% reduction in the use of more hazardous pesticides (EEA, 2023). The regulation contributes to the United Nation's Sustainable Development Goals (SDG2, food security; SDG3, good health; SDG6, clean water; SDG8, economic growth; SDG12, responsible consumption and production; and SDG15, protection of life on land). A recent study on pesticide residues in ten European countries found that 68% of samples had at least one residue. Also not approved compounds can still be found in samples (Silva *et al.*, 2023). The reduction in pesticide were analyzed in several models and resulted in a decline in production of 7.9% in cereals, 11% in oilseeds and 10.4% in vegetables (Schneider *et al*., 2023). In order to counteract the negative effects on crop productivity caused by reduced pesticide applications, biological plant protection products with a high and constant effectiveness are particularly important.

Biological products can act as biocontrol agents, bio stimulants or biofertilizers. Biocontrol agents can protect the plant in different ways: through direct antagonism against pathogens, such as parasitism, antibiosis and competition, or indirectly by inducing the plant's defenses. Screening for biocontrol activities is still challenging, as each method has his limitations. Plate assays have the advantage of his high throughput. However, only those control agents which produces secondary metabolites or volatile compounds can be found. Another possibility would be to use a microscope to determine whether a control agent damages the cell structure of a pathogen. However, this would again be done in an artificial system that does not correspond to the natural plant/ pathogen system. The use of the plant, either as a detached leaf or as a whole plant, is labor-intensive, but direct as well as indirect defense mechanisms can be found. Leaf assays are not always appropriate, as leaves with a thin wax layer in particular can be damaged by the biocontrol strain.

The starting point for a screening against plant pathogens are strains from a strain collection. The AIT strain collection contains around 6.000 isolates from different plant and soil sources. The collection covers a broad diversity of bacterial classes and genera and serves as a starting point for the screening of biocontrol activities.

Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* is one of the most serious leaf diseases in wheat. In order to search for control agents against STB, bacterial strains were selected from the AIT strain collection. Most of the selected strains belonged to *Bacillus*, but *Streptomyces*, *Pseudomonas* and *Paenibacillus* were also tested. In order to find a correlation between control and enzymatic activities, the strains were analyzed for chitinase, proteinase, lipase and cellulase activity as well as the ability to produce chitosan. However, none of the enzymatic activities could be correlated with the control activities in the experiments with detached leaves.

For the detached leaf assays the wheat leaves with the adaxial site up were sprayed with the control agent and one day later with a *Z. tritici* strain. After five days, the DNA of the leaves were extracted and the amount of *Z. tritici* were compared between the control and without control agents using qPCR. In total 45 bacteria were tested, and five strains reduced the amount of *Z. tritici* DNA and no *Z. tritici* DNA could be found in seven strains in the qPCR system

The positive strains belong to the genera of *Bacillus* and *Streptomyces*. Both genera are well known for there antifungal activity. *Streptomyces* consists of a group of Gram-positive, aerobic, non-motile, catalase positive, and non-acid-fast bacteria with a filamentous form that resembles fungi (Flärdh & Buttner 2009). The genome size of *Streptomyces* strains is usually large, *i.e.* between 8-9 Mbps. Members of *Streptomyces* are well-known for the production of bioactive compounds including antibiotics and strains with antifungal activities are known (Nazari *et al.*, 2023). Several products based on *Streptomyces* are commercially available to combat fungal diseases. One example is MYCOSTOP® (*S. griseoviridis* K61) from Lallemand Plant Care. This product protects plants from damping off disease by soil-borne pathogens. Another example is Actinovate® (*S. lydicus* WYEC 108) AG from Novozyme, a fungicide with a broad application range from soil to foliar application.

Bacteria of the group *Bacillus* are known to produce a wide range of antagonistic compounds of different structures. The most important bioactive molecules from the genus *Bacillus* are non-ribosomally synthesized peptides and lipopeptides, polyketide compounds, bacteriocins and siderophores. Most bacilli are able to form spores, which makes them more stress-resistant and therefore more suitable for agricultural applications. RhizoVital® 42 and AmyProtec 42 from Andermatt or Serenade® from Bayer are examples of commercial products based on *B. amyloliquefaciens* FZB42

Before a new strain can be used commercially, not only must its efficiency be proven, but its safety for humans and the environment must also be guaranteed. With the help of bioinformatics, it is possible to analyze genomes to detect gene clusters that could be harmful to humans or the environment. In *Streptomyces* in particular, there are many strains that produce clinically important antibiotics. To avoid any risk when using such strains, prior analysis of potential antibiotic clusters is essential.

The strains we found, are still being investigated to see whether it can combat other fungal diseases. Another important part will be to demonstrate its efficiency on whole plants in the greenhouse and in the field.

Keywords

Biocontrol · crop productivity · microbe · plant-microbe interaction · plant pathogen · *Streptomyces*

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Down in the underground: current approaches to study root-microbiome interactions *in situ* to enhance cereal yield

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Abstract

Worldwide, drought is a significant agricultural problem that can cause crop failure and decreased productivity. In response to climate change, breeding crops that are more resilient to biotic and abiotic stresses offers both opportunities and challenges. Crop breeding relies heavily on root phenotyping because a plant's ability to absorb water and nutrients is largely determined by the structure and function of its roots. For crop improvement and selection, trait-based root phenotyping is a crucial tool. Current plant breeding efforts target root trait adaptations that will result in new, more stress-tolerant crops and higher yields by enhancing the plant's ability to explore the soil and interact with beneficial microbial communities. Therefore, we aim to compare in five fields spread across three countries, with disparate soil and climate profiles, root-microbiome interactions depending on individual barley genomes. Images of about 50,000 individual roots from 571 different varieties of barley grown in the three countries were analysed in the first year of the project and we could distinguish between root traits that are primarily controlled by the plant's genome and those that are rather influenced by environmental cues.

Keywords

Barley · drought resilience · high-throughput root phenotyping · *Hordeum vulgare* · root traits

Further reading

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BIOSTIM: Comparison of plant biostimulants in a pot experiment

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Abstract

Beneficial soil microbes can have numerous positive effects on plants. Plant growth promoting rhizobacteria (PGPR) can influence a plant's nutrient supply, improve its disease resistance and stress tolerance and stimulate root development and nutrient absorption by production of phytohormones (Vessey *et al.*, 2003; Lugtenberg & Kamilova, 2009). Arbuscular mycorrhizal fungi (AMF) can benefit crop plants by mobilizing nutrients, enhancing root and plant growth, reducing stress and providing pathogen protection. In return, the plant supplies the fungi with carbon. However, the effects of this symbioses depend on various parameters (*e.g.* soil properties, bacterial community) (Smith & Read, 2008).

Several strains of PGPR and AMF are used as active ingredients in commercial plant biostimulants to improve crop performance under reduced fertilizer inputs. In the current study we investigated plant biostimulant products for their effects on the early development of winter wheat in different agricultural soils from Lower Austria and Burgenland. Reduction of the natural soilborne microbes by gamma irradiation was investigated to better understand the role of the autochthonous microbiome on added microbes from plant biostimulants.

Certified organic seeds of cv. 'Energo' were planted in sterilized and non-sterilized soils from four different locations (*i.e.* Absdorf, Lackendorf, Moosbrunn and Steinabrunn) exhibiting distinct properties in terms of pH, soil type and sand:silt:clay ratio. Soil sterilization by gamma irradiation was done by Mediscan (Seibersdorf, Austria) and distinct bacterial (BS4, BS6) and AMF (BS5) mixtures were applied on the seeds respectively in the soils before planting. Untreated seeds (BS7) were planted as a control and six replicates of the distinct microbial treatments and the control were planted in each of the soil samples. One replicate included six planted seeds and after the emergence of the plants, the number of plants was reduced to three in each pot.

Plants were put in a climate chamber where growth conditions were set to 16°C and 12 h light. Chlorophyll- and anthocyanin content were measured after two and three weeks after planting non-destructively with the Polypen 410 V-VIS device (Photon Systems Instruments, Drasov, CZ) and plant length was determined continuously. Destructive measurements at the end of the experiment (*i.e.* 25 days after sowing) included root length and area, and leaf area. These measurements were done with WinRhizo PRO 2013 software (Regent Instruments Inc., Québec, CA). Furthermore, dry root biomass and fresh and dry aboveground biomass were determined.

The results show that soil type has a statistically significant influence on all measured parameters, *i.e.* root length and area, leaf length and area, aboveground- and root biomass, and anthocyanin and chlorophyll content. Sterilisation has a statistically significant negative impact on root length (Fig. 1), but a positive influence on leaf length and aboveground biomass (+ ≈60%) for which differences became obvious after an initial lag phase of ≈10 days, where all nutrients were provided by the seed (Fig. 2).
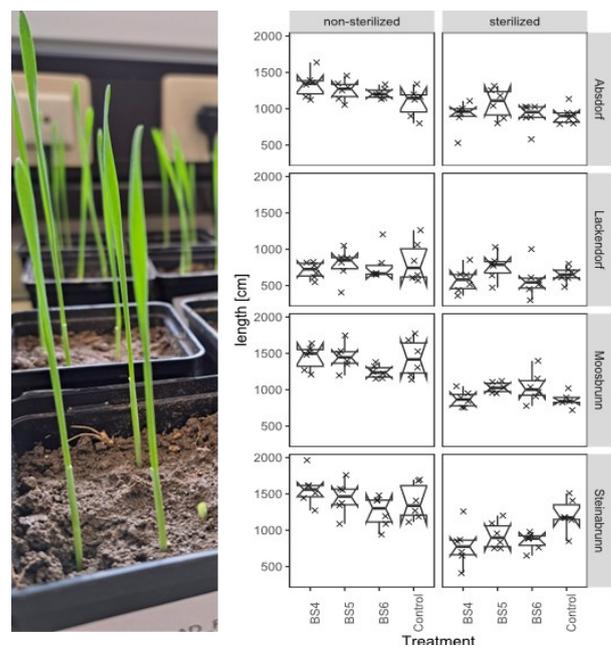
Figure 1: Effect of soil origin (Absdorf, Lackendorf, Moosbrunn, Steinabrunn), gamma irradiation and plant bio-stimulant treatments (BS4-BS6) on root length of winter wheat plantlets in the pot experiment.

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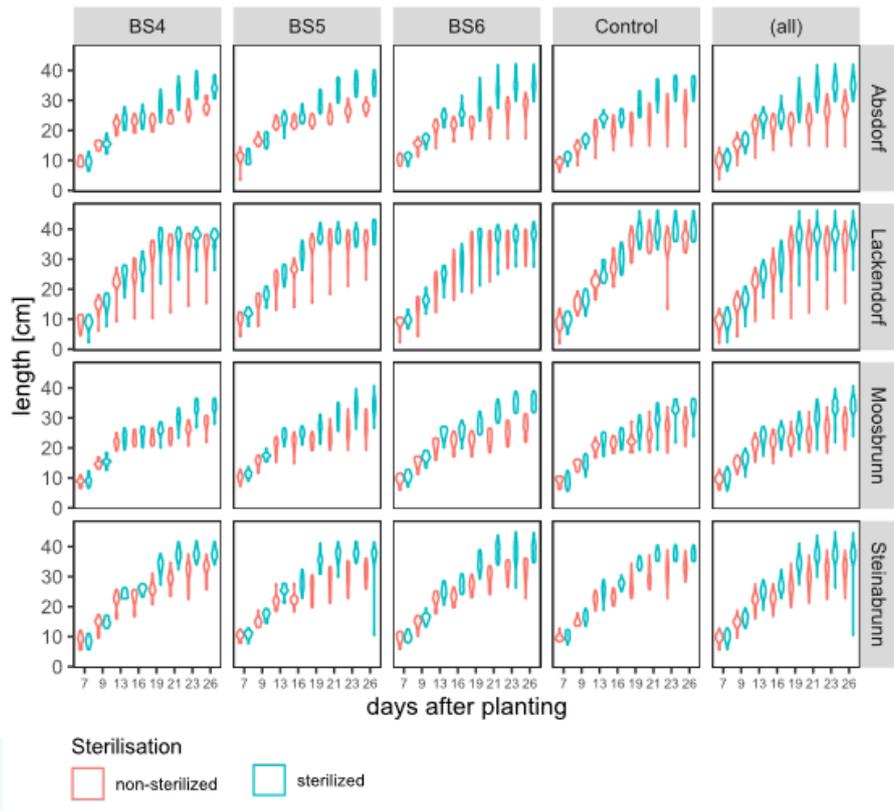
Figure 2 Variability in leaf lengths of wheat plantlets in gamma-irradiated and untreated soils of different origin after treatment with bacterial mixtures (BS4, BS6) and arbuscular mycorrhi-

Morever, plant biostimulants have no consistent effects on the measured parameters, but positive or negative effects could be observed in certain combinations of soil type and sterilisation. According to the literature, plant biostimulants are a promising approach for agriculture to manage certain challenges. However, the effect of biostimulants depends on various parameters and has to be analysed under controlled conditions before application on the field.

These findings indicate that soil sterilization and soil type can have an effect on plant growth, however, these factors do not correlate with the effect of the microbial treatments. Therefore, in order to understand the lack of effect, further research regarding the establishment of the added microorganisms is needed.

Keywords

Arbuscular mycorrhizal fungi · gamma irradiation · leaf length · plant biostimulants · plant growth promoting rhizobacteria · root length

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The breeder's eye - Reality or fairy tale?

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Abstract

Breeders claim to possess the ability to select higher-yielding lines than randomness could do. We conducted a trial to quantify this effect. To this end we randomly selected 100 lines of Breun's DH wheat program and compared their yield to the lines chosen by the breeders. The lines were selected in one year and tested for yield the following year at three different locations. The selection intensity ranged between 6.2% and 9.2% of the produced lines. We observed a yield advantage of the breeder's lines of 2.4, -0.2 and 2.6% for the years 2021, 2022 and 2023. We showed that treatment had no influence on this effect. The missing yield effect in 2022 appears to be caused by strong selection to disease resistance, especially Fusarium head blight (FHB) and later ripening of these lines. In the first two years, the best randomly selected lines were also selected by the breeder. Two of these lines were subsequently even applied in official trials in Germany.

Keywords

Breeding · selection intensity · *Triticum aestivum* · wheat · yield

Introduction

Wheat (*Triticum aestivum*) is one of the major crops throughout the world. In Europe, it is the most important self-pollinating cereal. Although farm saved seed is very important, wheat breeding is an interesting business to many breeding companies. Wheat breeders often claim to possess a "breeder's eye", which means they can distinguish good lines from bad ones. Since breeding for resistance to diseases, lodging and winter hardiness can also be done by people with no formal qualification for cereal breeding, we tried to determine whether there is an effect of a breeder on the yield of wheat lines compared to random selection.

Material and methods

Like most breeders, Saatzucht Josef Breun GmbH&Co. KG (Breun) uses the double haploid (DH) system as a part of its breeding programme. The DH lines are produced at the DH laboratory of the Bayerische Landesanstalt für Landwirtschaft (LfL) in Freising by intergeneric hybridisation with maize. The seedlings are planted in a field at Lenglern near Göttingen, harvested without selection and grown as observation plots at Herzogenaurach and Lenglern (if there was enough seed). In this study we selected DH lines the usual way for the Breun breeding programme and tested their yield in the years 2021-2023. The selection in 2021 and 2022 was done by Ludwig Ramgraber, in 2023 by Norman Philipp. In addition we selected about 100 lines per year randomly. As a breeder would not select combinations of the reduced height genes *Rht-B1b* and *Rht-D1b* because they are known as low-yielding, such combinations were also discarded from random selection. Table 1 shows the number of lines, selected lines, randomly selected lines and the overlap of both. For the lines that were only selected randomly, there was no maintenance breeding. The lines were tested for yield at three locations (Herzogenaurach, Morgenrot near Quedlinburg and Lenglern), depending on the seed quantity in one up to 6 replications, 0-3 of them without treatment and 1-3 replications treated with fungicides. In the optimum case each location had one replication untreated and treated. The Breun trial system is a standard trial method, where each 5th plot is a check variety.

Table 1 Selection intensity during the trial period 2021 - 2023

Period	A2	Selection breeder	%	Selection random	Selection breeder+ random
2020/21	5623	515	9.2	99	10
2021/22	5473	337	6.2	100	5
2022/23	6987	618	8.8	99	11

Results and discussion

Fig. 1 shows the medium yield of the lines selected by the breeder and those selected randomly. The mean number of replications is between 3.9 and 5. In 2021 and 2023, there was a positive effect of the selection by the breeder of 2.4% and 2.6%, respectively, whereas in 2022 there was no effect (-0.2%). The effect of selection by the breeder did not depend on resistance to diseases (Fig. 2). It was the same in the treated replications.

The missing effect in 2022, the year with the strongest selection by the breeder, can be explained by the influence of diseases, especially by the effect of FHB on high-yielding lines, which might

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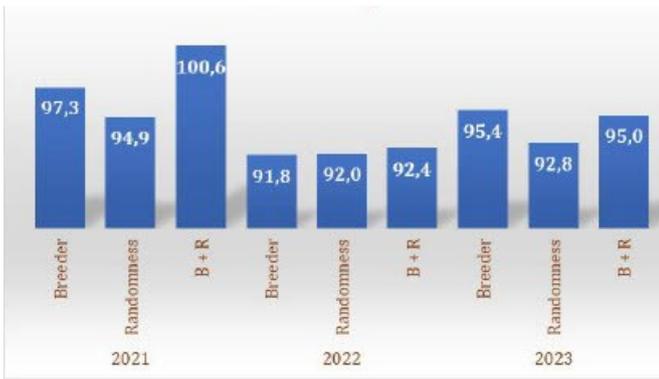
Figure 1 Grain yield relative to cvs. 'Informer' (2021-2023), 'Bosporus' (2021) and 'Floki' (2022-2023)
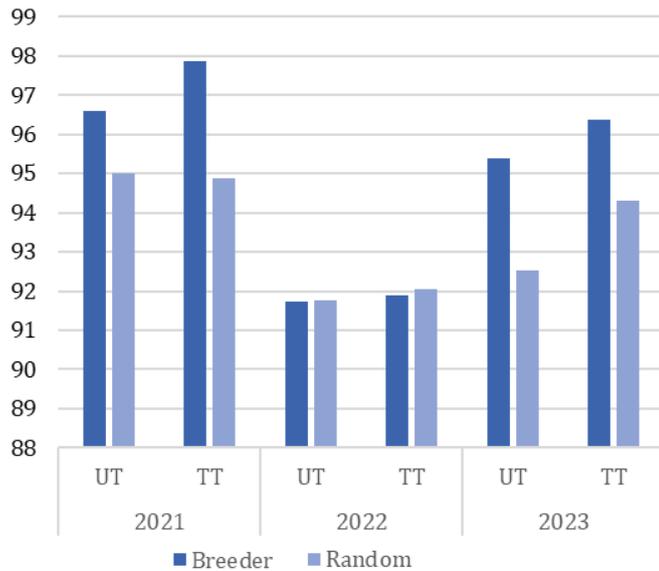
Figure 2 Relative grain yield of wheat breeding lines selected by the breeder or randomly in untreated (UT) or fungicide treated (TT) trials, 2023-2023.

have been discarded in the year 2021. Selection for resistant lines also favours late ripening. Early heading made a yield advantage of about 0.9 dt ha^{-1} and the lines selected by the breeder had a heading date of 0.8 days later than the randomly selected ones. In general, strong selection for disease resistance and other factors which do not draw benefit from the "breeder`s eye" naturally minimize breeder's influence.

In 2021, the three highest-yielding lines which were randomly selected were also chosen by the breeder. Two of these lines were even applied in official trials in Germany. In 2022, the best line also still exists in the Breun breeding system. In 2023, one line which was chosen randomly and by the breeder is prepared for application to official trials.

Overall, selection by the breeder proved to outperform random selection in terms of yield, but Breun applied lines which were also selected by chance. One reason for this last fact can be explained by the crossings chosen for DH production, which are generally combinations of promising new lines. With the rising importance of breeding for disease resistance and new breeding techniques such as Genomic Selection, perhaps finding the right cross is becoming more important than before.

The results reported in this study were collected in the trenches of practical plant breeding. We have not conducted a thorough statistical analysis of all the data. Colleagues working for other companies might observe stronger effects, for example caused by stronger influence of disease resistance, but maybe lower effects on treated yield because especially Herzogenaurach is a very selective location for high grain yield.

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30 years of quality wheat breeding at the breeding station Probstdorf

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Abstract

The breeding station Probstdorf is located in the middle of Europe at the western border of the Pannonian climate region with a continental influence. This provides good conditions to breed for the whole Pannonian Basin and the region along the river Danube from Vienna to the Black Sea.

When Austria prepared for joining the European Union, adaptations in wheat classification were made by Oberforster et al. (1993). A new scheme of quality groups was established, primarily based on baking volume. Groups 7-9 are quality wheats, 3-6 are milling wheats and groups 1-2 are wheats for feed or other use. When Austria became EU member in 1995, the Austrian agri-environmental program was started, which favoured strong limitations of nitrogen and agrochemical input. Especially in the Pannonian region of Austria, most farmers abstain from using fungicides in wheat production. Nitrogen input was limited to 130 kg/ha in this region for getting subsidies.

In 1993, 46 winter wheat varieties were released in Austria, 17 varieties belonged to the quality wheat groups 7-9, which can be regarded as "improver wheat" varieties in an international context. Before EU membership, the Austrian wheat market was highly regulated, the eastern part of Austria was dedicated to the production of only quality wheat. This was continued to large scale on voluntary basis until nowadays. In 2023, 70 winter wheat varieties are released and described in Austria (ÖSL 2023), 59% of all Austrian varieties are quality wheats. 30 out of the 41 currently listed quality wheat varieties, but only 3 out of 29 milling or feed wheat varieties were bred at Probstdorf.

The Austrian Exchange for Agricultural Products in Vienna (Börse, 2023) defines that both 'quality wheat' (protein content ≥14%) and 'premium wheat' (protein content ≥15%) must be varieties of quality group 7 or higher. This regulation has a strong impact on variety release, in case a breeding line does not reach quality group 7, there is practically no chance to be marketed in Austria. Therefore most of these breeding lines were withdrawn by the breeding companies before release during the last 30 years.

In 2000, the company Saatzucht Donau was founded as a merger of the breeding activities of Probstdorfer Saatzucht and Saatbau Linz. Early-generation quality wheat breeding material developed by Anton Neumayer at Saatbau was joined into the Probstdorf

programme. Wheat variety 'Arnold' is an example from this fruitful collaboration.

In 2001, Austria was the first country in Europe to start with a solely organic value for cultivation and use (VCU) test for wheat. National and international research projects supported the implementation of "breeding for organic agriculture" (Löschenberger et al., 2008; Miko et al., 2014; Muellner et al., 2014). Due to subsidies for organic farming, favourable climatic conditions in eastern Austria and the availability of adapted varieties, organic wheat seed sales comprised 20% of all Austrian wheat seed sales in 2020.

When Hungary, Slovakia, Slovenia, Poland, Czech Republic, Estonia, Lithuania and Latvia joined EU in 2004, seed exchange was eased, this gave a great opportunity to extend activities that had started in the mid 1990's.

Numbers of Saatzucht Donau (SZD) wheat variety releases from Probstdorf between 2003 and 2023 are: 52 in Austria, 46 in Hungary, 40 in Slovakia, 33 in Ukraine, 19 in Romania, 17 in Croatia, 6 in Canada, only 2 in France and only one variety in Germany (https://www.saatzucht-donau.at/sorten/). Before foundation of SZD, an important Probstdorf variety with the highest number of foreign releases was 'Ludwig', released in seven countries including Germany, and bred by Ludwig Seibert, wheat breeder at Probstdorf until 1995.

From 1951 to 1988, Hermann Hänsel had been wheat breeder at Probstdorf. His idea about a flexible breeding programme highly influenced breeding during the last 30 years: Hänsel favoured a continuous interaction between breeder and breeding programme, not a fixed breeding procedure. Hänsel treated the crosses differently according to their behaviour during the years, in a holistic approach. He favoured genotype by environment by breeder interaction. The question remains whether it is possible to select for more stable or more resilient varieties by any kind of an artistic approach, by wandering through the nursery in an open-minded, curious manner, seeking for exceptional wheat types.

„My relationship to specific plots that caught my attention was indeed several times decisive for variety development". Hänsel describes his own „questionism" and the dedication to be a breeder. For decisions, there is the necessity of a continuous flow of filtering knowledge, of ideas and thoughts" (Hänsel, pers. commun.).

The Austrian National Descriptive List of Varieties (AGES, 2023) describes 32 to 35 traits for each wheat variety. Apart from agronomic characteristics, the description of quality parameters, detailed dough rheology parameters from faringram and extensogram and the additional classification of export quality by alveogram has outstanding importance for farmers, traders, millers and bakers. Enhancing variety awareness and knowledge of specific quality characteristics is a key element to secure high wheat commodity quality. Approximately one third of Austrian wheat production is exported, mainly as improver wheat. Export of organic wheat is supported by the combination of favourable climatic conditions and the availability of adapted high quality wheat varieties.

Selection for quality throughout the breeding process
Strong selection for quality starts with scoring the grains of single ears, this is done in all early generations. As soon as enough material is available, near infrared spectroscopy (NIRS) predicts a range of quality traits in addition to protein content (Löschenberger, 1993). NIRS of the grains of single ears was discontinued due to inefficient workload. Calibrations of NIRS devices were developed during 30 years, additional parameters were introduced, such as baking volume, grain hardness, flour water absorption, etc. In the latest versions faringram stability and alveogram W-value are also predicted. Anyhow, we are aware that most quality traits are highly related to protein content and too strong selection for quality mostly means selection against high yield and vice versa (Dallinger *et al*., 2023). On the other hand, we found that NIRS provides valuable additional quality information apart from only protein content.

A simplified Zeleny sedimentation test that was developed by Hänsel in the 1960's works without tempering samples before milling. This sedimentation value was the main selection criterion at Probstdorf for decades, especially before the introduction of NIRS. Micro-SDS sedimentation test was introduced in 2000 by Julia Lafferty and since then, together with NIRS protein content partly replaced the Zeleny sedimentation test in the early generation material such as F_4 or doubled haploids (Dick & Quick, 1983). Samples of advanced material are routinely sent to the Versuchsanstalt für Getreideverarbeitung (https://vg.or.at/) for analysis of faringram, extensogram and alveogram. Based on these results, genomic selection (GS) was introduced for quality improvement (Michel *et al.,* 2016). Despite interesting results (Michel *et al.,* 2017a), glutopeak analysis was applied for selection only after 2020, when SZD had purchased its own device. Meanwhile, Brabender published new protocols, such as the "Rapid flour check", which in our case worked satisfactorily only with samples from our organic test site having lower protein contents. As we work with breeding material grown at very diverse organic and conventional test sites (Table 1), 9 g of flour from the Brabender Junior mill and 10 mL of water turned out to give interpretable results in most cases. This protocol was developed by Almuth Müllner. In case of very high protein content and very high water uptake, *e.g.* due to high temperatures at ripening, we used 8 g of flour instead of 9 g and adjusted the results accordingly by adding 10 GPU (Glutopeak units) to the A2 value to get comparable results with the SZD standard procedure.

Genomic and phenomic predictions
Table 2 shows the correlation coefficients of extensograph flour water absorption with diverse NIRS estimates (protein content, water uptake and grain harness), glutopeak indices A2 and A35 and A4, two sedimentation values as well as genomic prediction (GS). We are aware that genotype by environment interaction is included in all comparisons except in genomic prediction, as analysis was done on identical samples within sample year and system - whereas GS model based on previous years' results created one value per genotype as a forward prediction. GS was only slightly below phenomic prediction by NIRS. Anyhow, Glutopeak A2 was the clearcut best predictor for extensograph water absorption, on the other hand NIRS estimate for water absorption is much faster and thus cheaper and comes together at the same time with protein content. As all predictions show uncertainties, phenomic prediction, glutopeak measurement and genomic prediction are used together in parallel, following the idea of Michel *et al.* (2017b) introducing "genomics assisted selection". Existing data from breeding can be used to solve newly arising questions - we appreciated the approach by S. Michel to scientifically analyse "real breeding data" and draw new conclusions.

What is the breeder's role - do we still need to go to the field?
Knowing the genetic material, the breeder constantly controls whether field plots are correct. Monitoring genetic diversity of the material can be done by genetic analysis, but field observations give additional impression about genotype by environment interaction. The breeder may be inspired for specific crosses by inspecting the plots having in mind knowledge about the characteristics of specific material. Selection for homogeneity and purity can almost only be done in the field. When working with GS we constantly try to avoid too many „self fullfilling prophesies", a constant „questionism" is needed, we constantly need to update our models. In order to reduce the risk of GS, we recommend to combine genomic and phenomic selection.

Follow your ideas - there is still need for intuition - „Breeding is half art half science" (H. Hänsel)

Table 1 Quality results, 2019-2022, of project TRIBIO: range and mean of grain protein content of samples from conventional locations (CON) Probstdorf (2019) and Weikendorf (2020-2022) and organic location Dörfles (ORG) which is located close to Weikendorf.

	2019 CON	2020 ORG	2020 CON	2021 ORG	2021 CON	2022 ORG	2022 CON
Samples (n)	150	89	112	136	136	136	136
Range (%)	10.2-16.5	7.8-11.6	10.9-17.1	9.0-15.0	12.1-16.7	11.0-15.7	12.8-17.6
Mean (%)	13.6	9.5	14.3	11.0	14.4	12.5	15.1
Difference CON – ORG (%)			4.8		3.4		2.6

Table 2 Project TRIBIO: extensograph water absorption and Pearson correlation coefficient for diverse traits: NIRS protein content, water absorption and grain hardness, glutopeak indices A2, A35 and A4 (2750 rpm, 36°C, 9 g flour, 10 mL water), modified Zeleny sedimentation and micro-SDS-sedimentation, as well as genomic forward prediction GS for 2021 and 2022 and the range of water absorption for each sample set.

Trait	VG19-CON (n=150)	VG20-ORG (n=89)	VG20-CON (n=112)	VG21-ORG (n=136)	VG21-CON (n=136)	VG22-ORG (n=136)	VG22-CON (n=136)
Protein content NIRS	0.47	0.27	0.40	0.20	0.18	0.24	0.37
Water absorption NIRS	0.55	0.53	0.55	0.61	0.52	0.61	0.46
Grain hardness NIRS	0.53	0.63	0.14	0.62	0.47	0.55	0.44
Glutopeak A2	0.85	0.79	0.63	0.81	0.87	0.82	0.60
Glutopeak A35	0.64	0.66	0.58	0.65	0.55	0.34	0.47
Glutopeak A4	0.65	0.61	0.60	0.53	0.66	0.59	0.48
Zeleny sedimentation		0.40	0.06	0.28	0.13	0.37	0.28
SDS sedimentation		0.42	-0.20	0.13	-0.04	0.12	0.01
Genomic Prediction GS				0.52	0.54	0.53	0.36
Water absorption sample range	50.0-61.0	49.7-58.6	54.0-63.7	49.4-59.0	50.8-58.4	51.3-61.0	51.6-60.8

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Is nitrogen use efficiency neglected in registration trials?

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Abstract

Nitrogen is an essential nutrient in crop production and human nutrition. The tremendous increase of global food production in the past was possible mainly by increasing the input of nitrogen fertilizer due to synthetically produced nitrogen. More recently, application of growth control chemicals and the introduction of short straw genotypes allowed for higher nitrogen fertilization. However, adverse environmental impact increased with more intensive cropping systems. Actually, the total N input in Germany is about 201 kg N per ha on agricultural land while the total output is 69% and the surplus budget is 31% of total input. EU policiy measures aim towards a reduction of N surplus by 50% and N fertilizer use by 20%. But at the same time an increasing world population needs higher food production. Therefore, improved varieties with higher yield are needed, however with less nitrogen use, i.e., higher nitrogen use efficiency (NUE). NUE is the result of a complex and dynamic process of the interaction between soil, plant and environment. NUE is considered as the ratio between harvested products and N inputs. We asked the following questions: Was breeding progress achieved for NUE and related traits in registration trials? How closely linked are grain yield (GYLD) and grain protein concentration (GPC%) with nitrogen yield accumulated in grain (NYLD)? How large is the heritability of NUE and related traits? Is there a potential to improve NUE in registration trials?

Based on long-term variety trials of cereals and winter oilseed rape across a wide range of environmetal conditions in Germany, we quantified long-term breeding progress for NUE between 1995 and 2021. As N input we used the available N (kg ha^{-1}) corresponding to the sum of mineral and organic N fertilizer and the soil mineralized N (N$_{min}$) assessed in spring. As traits were evaluated GYLD (dt ha^{-1}), GPC (%), NYLD (kg N ha^{-1}), NUE for GYLD (kg N per kg N) derived as GYLD per kg of available N per ha and NUE for NYLD (kg N per kg N) derived as NYLD per kg of available N per ha. Trials were treated according to crops good local agronomic practice, depending on the yield potential of the trial site. All varieties in an individual trial received the same treatment. Only varieties with at least two-year results were used. One to three trial series were grown each year per crop. In S1, S2 and S3 varieties in the first, second and third testing year were included. For spring wheat only one trial series per year was conducted. The testing period was three years corresponding to one testing cycle. Breeding progress was estimated by a mixed linear model including a time trend represented by a quadratic regression function with the harvest year as covariate. The breeding progress was derived as the difference of the trend function at year 2021 and 1995, which comprised the progress due to new varieties and non-genetic causes, like fertilzer effects. We further decomposed the correlation coefficient between two traits according to the genotypic, genotype × environment interation, evironmental and residual model effects to show the strength of association for individual effects between traits. Further, we estimated heritabiliy coefficients for variety means over all trials within the three-years testing period, because traits usable for variety registration should have a reasonable repeatability or predictive power.

In Table 1 the breeding progress between 2021 and 1995 is shown as absolute and relative values, based on trend level 1995. Considerable reductions of N fertilzer rates in the range of 5.7% for winter wheat to 32.4% for spring wheat occurred, except in spring barley N ferlizing increased by 16.8%. For NYLD, however, no significant reduction was estimated despite less N fertilizer input. GPC reacted more sensitive to decreasing N rates, especially winter rye and seed rape varieties reacted with decaying GPC while for spring barley and wheat no loss was estimated. Contrary to GPC, GYLD still increased up to nearly 13.7% for winter rye hybrid varieties. Only spring wheat reacted with a slight decay for GYLD, however by strongly reduced N fertizer. A strong increase for NUE of GYLD in the range 12.2% to 30.8% was achieved, while NUE of NYLD increased also, but not as strong as for GYLD. For NYLD, NUE also increased, but not as clear as for GYLD. A noticably low NUE of winter oil seed rape compared to cereals was found. Results of correlation coefficients indicated that NYLD was more strongly positively correlated with GYLD than with GPC.

Laidig F, Feike T, Lichthardt C, Schierholt A, Piepho HP (2023) Is nitrogen use efficiency neglected in registration trials? In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 43-44. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5

Table 1 Performance levels of time trends for breeding progress at years 1995 and 2021 and differences between levels 2021 and 1995 expressed in absolute (Diff) and relative (%) values based on level 1995.

Crop	N yield in grain kg ha^{-1}					N fertilizer kg ha^{-1}				
	1995	2021	Diff	%	sig	1995	2021	Diff	%	sig
Winter wheat	187.4	194.0	6.6	3.5	ns	180.1	170.3	-9.8	-5.4	ns
Winter rye hybrids	134.1	135.1	1.1	0.8	ns	130.4	107.8	-22.6	-17.3	***
Spring wheat	160.9	155.0	-5.9	-3.6	ns	163.1	115.9	-47.2	-28.9	***
Spring barley	94.3	102.3	8.0	8.5	*	68.6	83.0	14.3	20.9	***
Winter oil seed rape	131.4	131.6	0.1	0.1	ns	176.9	143.5	-33.4	-18.9	***

	Grain protein conc. %					Grain yield dt ha^{-1}				
	1995	2021	Diff	%	sig	1995	2021	Diff	%	sig
Winter wheat	13.1	12.5	-0.6	-4.4	**	95.4	103.3	7.9	8.3	**
Winter rye hybrids	10.5	9.3	-1.2	-11.2	***	84.9	96.6	11.6	13.7	**
Spring wheat	14.0	13.9	0.0	-0.2	ns	76.8	74.4	-2.3	-3.0	ns
Spring barley	10.8	10.3	-0.5	-4.5	ns	64.0	72.4	8.4	13.2	**
Winter oil seed rape	18.8	17.4	-1.4	-7.6	**	44.8	48.6	3.8	8.5	*

	NUE of N yield in grain kg kg^{-1}					NUE of grain yield kg kg^{-1}				
	1995	2021	Diff	%	sig	1995	2021	Diff	%	sig
Winter wheat	0.84	0.91	0.07	8.0	*	43.0	48.5	5.5	12.8	***
Winter rye Hybrids	0.89	1.01	0.12	13.0	*	56.7	71.9	15.2	26.8	***
Spring wheat	0.76	0.88	0.12	15.9	*	36.2	42.4	6.2	17.1	*
Spring barley	0.76	0.83	0.06	8.0	ns	51.9	58.9	7.0	13.5	*
Winter oil seed rape	0.66	0.77	0.12	17.9	***	20.9	27.3	6.4	30.8	***

Further, the weak to moderate positive genotypic correlation of GYLD and GPC with NYLD indicated that selection for high NYLD does not negatively influence the selection for high GYLD or GPC. In addition, the decomposed correlation coefficients showed that the rank of genotypic values of GYLD and NYLD is equivalent to the rank of NUE for GYLD and NYLD. This important result reveals that selection for high GYLD or NYLD is equivalent to selection for high NUE. Heritability of variety means for crop's typical number of trials within a varieties testing period showed that GPC had the highest heritability coefficients across crops, while heritability of NYLD, GYLD and NUE of GYLD was of about of the same magnitude, indicating that NYLD is equally reliable as GLYD, which is the most important registration criterion. This suggests, that NLYD could be used as criterion for registration trials. Our study showed that considerable breeding progress in GYLD and NUE was achieved in the last 27 years despite strong reduction of N fertilizer, further that NUE was not neglected and that NYLD showed a reasonable potential to be used in registration trials to improve NUE and reduce adverse environmental impact.

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Wheat breeding for organic farming: Is there a need for specific organic breeding programmes?

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Abstract

The question if a special breeding programme is needed for organic farming is not new but was already discussed 20 years ago at the 21st Getreidetagung in Detmold. Two arguments dominated at that time: (i) conventional breeding also covers the needs of organic farming and there is no need for a separate breeding programme for organic farming; (ii) conventional breeding only partially covers these needs and therefore an independent organic breeding programme is necessary. In recent years there have been increasing calls for purely organic breeding programmes for organic farming. However, these demands have no scientific basis.

Messmer *et al*. (2018) made a classification of the various forms of breeding. **Conventional breeding** as the status quo includes all varieties that have been selected under conventional management. This involves the use of seed treatment and herbicides, as well as ensuring an optimum supply of nutrients. The breeding objectives are geared towards conventional agriculture and only released commercial varieties are then tested for their suitability for organic cultivation. **Breeding for organic farming** is a product-oriented approach. The breeding objectives are focused towards the needs of organic farming, even if only some parts of the selection and the final multiplication steps take place under organic management. In contrast, **organic plant breeding** is process-oriented. Breeding is designed exclusively and specifically for organic farming. All selection and multiplication steps are carried out under organic conditions and the breeding methods are in agreement with principles of organic farming (Messmer *et al*., 2015) .
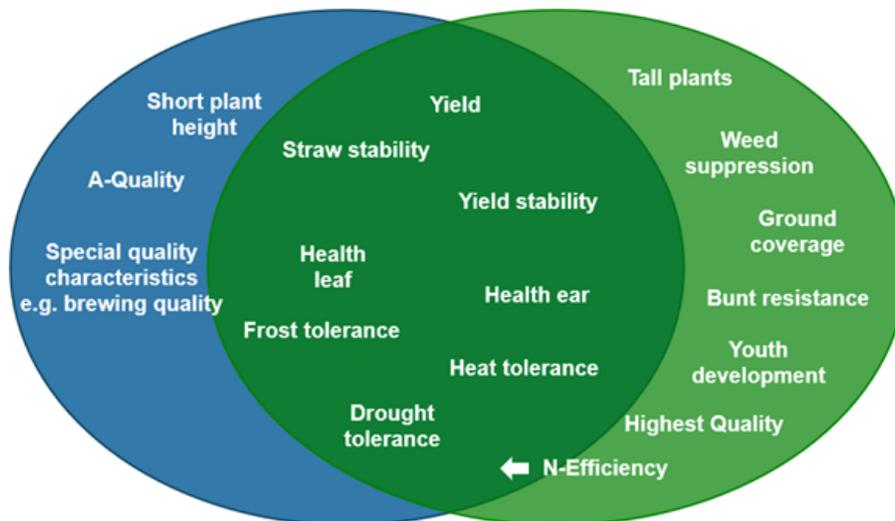
The breeding objectives of a breeding programme are based on the target group. Looking at breeding objectives of conventional and organic farming, it is noticeable that both cultivation systems have a large overlap of breeding objectives (Fig. 1). This overlap includes grain yield, yield stability, lodging tolerance, resistance against leaf and ear diseases, frost, heat and drought tolerance. But there are also breeding targets that were considered as purely organic ones until recently. For example, N use efficiency.

However, the EU and federal government regulations have reduced the maximum amount of fertilizer that can be applied. The plants must now be able to convert the lower quantities of applied N more efficiently into yield and quality, in form of grain crude protein and wet gluten content.

Despite many common breeding objectives, organic farming needs to pay particular attention to specific challenges. Weed suppression is one of them. Weed competitiveness is a complex combination of traits such as plant height, ground cover and juvenile growth.

The biggest difference between organic and conventional varieties is plant height. A comparison of commercial varieties shows that conventional varieties are on average 13 cm shorter than varieties for organic cultivation. It is reasonable to assume that tall varieties also have a higher degree of ground coverage. However, a comparison of conventional and organic varieties from 2013 to 2023, which were tested in national organic trials, shows no significant difference for this trait between the varieties derived from the different breeding programmes.

Probably the greatest challenge for organic farming and breeding is achieving a high baking quality. Baking quality is also a compley trait made up of the following characteristics: baking volume, dough elasticity and surface quality, falling number, sedimentation value, water absorption and flour yield (T 550). Baking volume is positively influenced by increasing wet gluten (R^2=0.58) and protein contents (R^2=0.62). Wet gluten and protein content are negatively correlated with grain yield. In multi-year (2013-2023) national organic trials, varieties from conventional breeding show higher yields than those from organic programmes. The majority of varieties that realise high yields under conventional farmings also do so under organic conditions. However, conventional varieties are inferior to organic varieties in terms of quality, whereas varieties bred for organic farming can keep up with the quality level of organic varieties which were selected and tested under organic conditions all over their development.

Figure 1 Comparison of breeding objectives between conventional (blue) and organic breeding (green) and their overlap (dark green).

As in any market, the question if special organic breeding programmes are needed is also a question of demand. The seed multiplication areas per variety and management conditions (conventional or organic) published by the Federal Plant Variety Office provide a good indicator. Fig. 2 shows the share of seed multiplication areas according to the above-mentioned breeding systems (conventional, conventional for organic, organic). Conventional varieties account for the majority (61.9%), followed by varieties bred for organic cultivation (19.4%) and organic varieties (18.7%). The market thus confirms what scientists have already recognized in several studies: varieties bred under conventional conditions are generally not unsuitable for organic farming (Grausgruber, 2009). One reason for this is that many agronomic traits, *e.g.* resistance to diseases, have high heritabilities and can therefore also be selected under conventional conditions. However, traits with low heritability, such as quality, should be tested under organic conditions (Lammerts van Bueren *et al*., 2011; Rakszegi *et al*., 2016). Muellner *et al*. (2014) concluded that the specific adaptation to a region or country is more important than the adaptation to a cultivation system.

A commercially sustainable breeding programme for organic and low-input agriculture should combine information from different input levels and different regions. In the whole discussion, it should not be forgotten that conventional varieties make an important contribution to genetic diversity in organic farming (Wolfe *et al.*, 2008). If organic-specific breeding objectives such as weed suppression, good baking quality at low N supply and testing of suitable lines under extensive and/or organic conditions are taken into account, conventional breeding can also provide good varieties for organic farming. However, these varieties should be tested in the organic national trials to demonstrate their suitability for organic farming. In conclusion, from an economic and scientific point of view, there is no need for an independent, purely organic breeding programme.
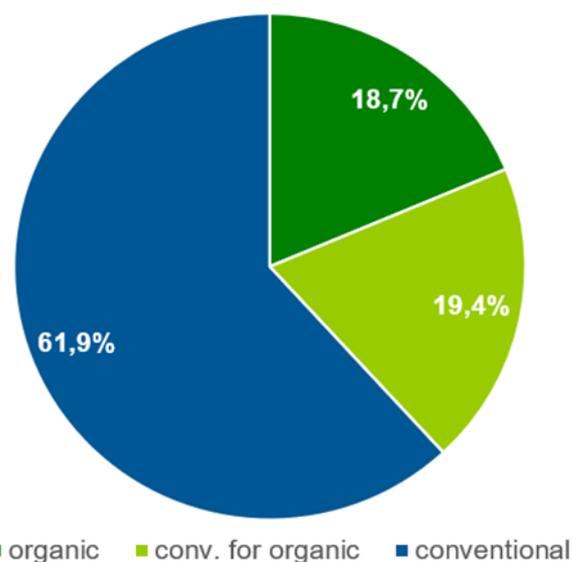
Figure 2: Share of commercial varieties developed by different breeding programmes of the total organic wheat seed multiplication area in Germany in 2023.

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Wheat yield and quality under conventional and organic farming

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Abstract

Wheat is the most important organic crop with the biggest diversity of organic products available (Devīte *et al.*, 2021). A wide range of diseases and abiotic stresses are the most limiting factors for wheat growing. Resistance to these stresses plays an important role in breeding for yield stability, the most desired trait among growers worldwide. The aim to provide enough food for an increasing world population, along with climate change, particularly the increase of global average temperature, more frequent periods of extreme heat, cold, heavy rainfall, or drought, as well as the threats of new diseases, are key challenges for the future (Javadinejad *et al.*, 2021; Reynolds & Braun, 2022). Organic farmers need varieties that are more plastic to specific regional environmental conditions, and able to achieve balanced yield and quality even under variable soil conditions (Arora, 2019).

The field experiment presented in this paper was conducted over three years (2021-2023) in four locations in the Czech Republic. Two of the sites (Domanínek and Uhříněves) were under organic (ORG) management, two (Stupice and Úhřetice) under conventional management (CON). Stupice, Úhřetice and Uhříněves are characterized by a warm, slightly wet climate and highly fertile soil suitable for wheat growing. On the contrary, Domanínek is located in a slightly warm, wet and not very productive region, and with an altitude of 350 m a.s.l. located higher than the other test sites. Nineteen spring wheat (SW) and sixteen winter wheat (WW) varieties were sown in a randomized complete block design with two replications and a plot size of 10 m².

Varieties 'Izzy' (SW) and 'Sultan' (WW) are included in the Czech list of recommended varieties for organic farming and were, thus, chosen as check varieties. Quantity and quality parameters such as yield, plant height, protein content, test and thousand-grain weight (TGW) were measured. In a first analysis, the overall comparison between ORG and CON sites was made. However, due to significant differences in agrometeorological conditions in Domanínek compared to the other three sites, and thus unbalanced average parameters between organic experiments Domanínek and Uhříněves, a further analysis compared only the trials from Stupice and Uhříněves. Both sites are located five kilometers from each other and are therefore influenced by similar meteorological conditions. Moreover, they are both characterized by highly fertile land.

Figure 1 Conventional field experiment in Stupice, Czech Republic.

Mean values of traits for 2021-2023 from both systems are given in Table 1. In general, most parameters were lower in the organic system than in the conventional. Mean grain yield was 35% (SW) and 29% (WW) lower than under conventional management protein content was 4% (SW) and 15% (WW) lower. Plant height was reduced under organic management by 4% for both SW and WW, and TGW was 4% (SW) and 3% (WW) lower. No difference between management systems was observed for test weight of SW, whereas for WW mean test weight was 1% lower in ORG. Table 2 shows the data for SW and WW in Uhříněves and Stupice. Mean grain yield in the organic site Uhříněves was 12% and 25% lower compared to conventional site Stupice for spring wheat and winter wheat, respectively. Protein content was 7% (SW) and 11% (WW) lower in Uhříněves. In contrast, plant height was 3% (SW) and 7% (WW) higher in the organic system than in the conventional. This is a result of the usage of plant growth regulators in conventional trials. TGW was 3% lower in SW and WW, test weight was 2% lower in SW and there was no difference for WW. The comparison between Stupice and Uhřínves shows that organic farming in fertile regions has a potential and the gap between CON and ORG mean grain yield could be lower than in national results where the average organic wheat grain yield in Czechia is 50% lower compared to the average grain yield of conventionally managed fields (ÚKZÚZ, 2023). In Czechia, many organic farms are located in the lower productive regions, similar to Domanínek and thus the average gap between the two management systems is

Table 1 The average mean of parameters, ORG and CON, years 2021 - 2023

System and type of wheat	Yield (t ha⁻¹)	Plant height (cm)	Protein content (%)	Test weight (kg hL⁻¹)	TGW (g)
Organic SW	5.5	87	13.7	77.6	39.7
Izzy	6.6	88	12.5	76.3	39.6
Conventional SW	8.5	91	14.2	77.8	41.3
Izzy	9.0	92	13.6	75.8	41.6
Organic WW	7.0	101	12.1	77.8	42.2
Sultan	8.2	97	11.5	78.2	42.4
Conventional WW	9.8	105	14.3	78.9	43.3
Sultan	10.0	102	13.7	79.2	41.7

Table 2 The average mean of parameters in Stupice and Uhříněves, years 2021 - 2023

System and type of wheat	Yield (t ha⁻¹)	Plant height (cm)	Protein content (%)	Test weight (kg hL⁻¹)	TGW (g)
Organic Uhříněves SW	7.4	89	13.9	77.2	40.2
Izzy	8.2	91	13.4	75.7	39.8
Conventional Stupice SW	8.4	86	15.0	79.1	41.5
Izzy	8.4	87	13.8	77.2	41.1
Organic Uhříněves WW	9.3	108	12.4	79.3	42.3
Sultan	10.3	105	11.7	78.9	41.9
Conventional Stupice WW	12.4	101	14.0	79.4	43.6
Sultan	10.7	102	13.7	79.2	41.3

high. Among the tested winter wheat varieties and breeding lines under organic conditions, ST 1518/19 stood out with the following parameters: mean grain yield of 8.2 t ha⁻¹, protein content 12%, plant height 103 cm, test weight 79.4 kg hL⁻¹ and TGW 40.2 g. Protein content and test weight were even higher than the average of the check variety 'Sultan', and thus ST1518/19 was submitted for variety registration.

Keywords

Breeding · conventional agriculture · organic farming · protein content · *Triticum aestivum* · variety testing

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The effects of the management system on the performance of a diverse winter durum variety set in Hungary

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Abstract

Durum wheat (*Triticum turgidum* ssp. *durum*) is the second most widely cultivated *Triticum* species after bread wheat (*T. aestivum*) in the world. In 2021, 33.8 million tons of durum grain were produced worldwide. North America is of particular importance, as the largest acreage of durum wheat is currently grown in Canada. Italian-style pasta produced from durum wheat is primarily processed by the dry pasta industry, however, couscous, previously known as a food of the North African people, is gaining more and more importance. Although durum wheat was originally a grain of the Mediterranean countries, today it has taken its place in the Hungarian crop structure. In 2022, durum wheat was cultivated on around 35 000 ha in Hungary. The acreage under organic farming is constantly growing and the consumers' demands for organic products are also increasing.

The objective of this study was to identify phenotypic parameters and yield components of the ECOBREED durum wheat variety set originated from different geographic sites in Hungarian organic and conventional management systems in three growing seasons. Between 2019 and 2022, unlikely the previous years' average, extreme weather conditions were observed. Durum wheat germplasm was examined in organic, common conventional and low-input conventional trials with 3 replicates on 6 m² plots. No fungicides were applied in any trial, however, conventional plots were treated with herbicide. During the growing season, early spring ground cover, heading time, plant height, disease severity and lodging were recorded. After harvest, grain yield, grain size (width, length), test weight (TW) and thousand kernel weight (TKW) were determined.

Analysis of variance revealed statistically significant effects for the year, management, genotype and their interactions for heading date, lodging, yield, grain width and length, TW and TKW. In the case of plant height and ground cover, the genotype × management interaction was not significant, which shows the strong genotypic determination of these traits.
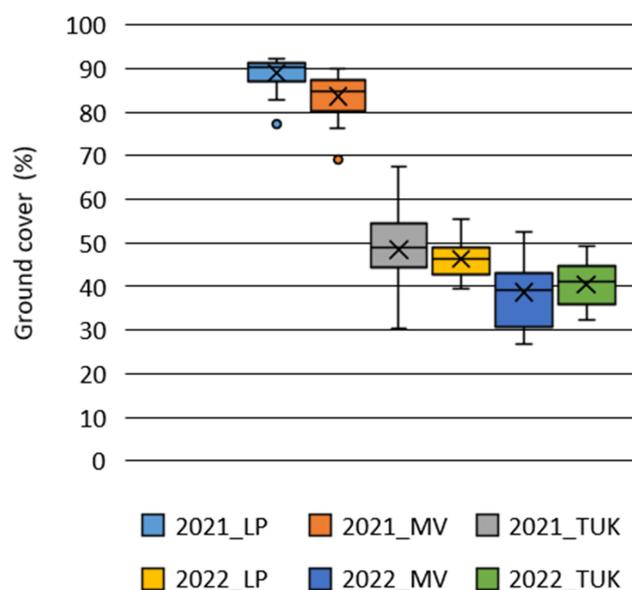
Figure 1 Variability in early spring ground cover of the durum wheat genotypes across years and management systems (LOW=low input, CON=conventional, ORG=organic; 2021-2022, Martonvásár, Hungary).

Analysis of the ground cover surface data (measured by Canopeo App) showed that the values of the conventional sites in 2021 were markedly different from the values of the organic condition, in contrast to the the drier year 2022 for which only slight differences were shown (Fig. 1). Heading date was significantly influenced by the year. In 2020 and 2022, the latest heading variety 'Mv Makaróni' headed on 20th and 21st May, respectively, while in 2021, due to the rainy and cold spring weather, 85% of the varieties started to head only after 20th May (Fig. 2a). Lodging was observed only on the conventional sites during two years. The average yield of the experiment was between 5.79 and 6.02 t ha⁻¹. The varieties 'NS-Zad', 'Sambadur' and 'Mv Vékadur' performed in each trial in each year significantly superior compared to the
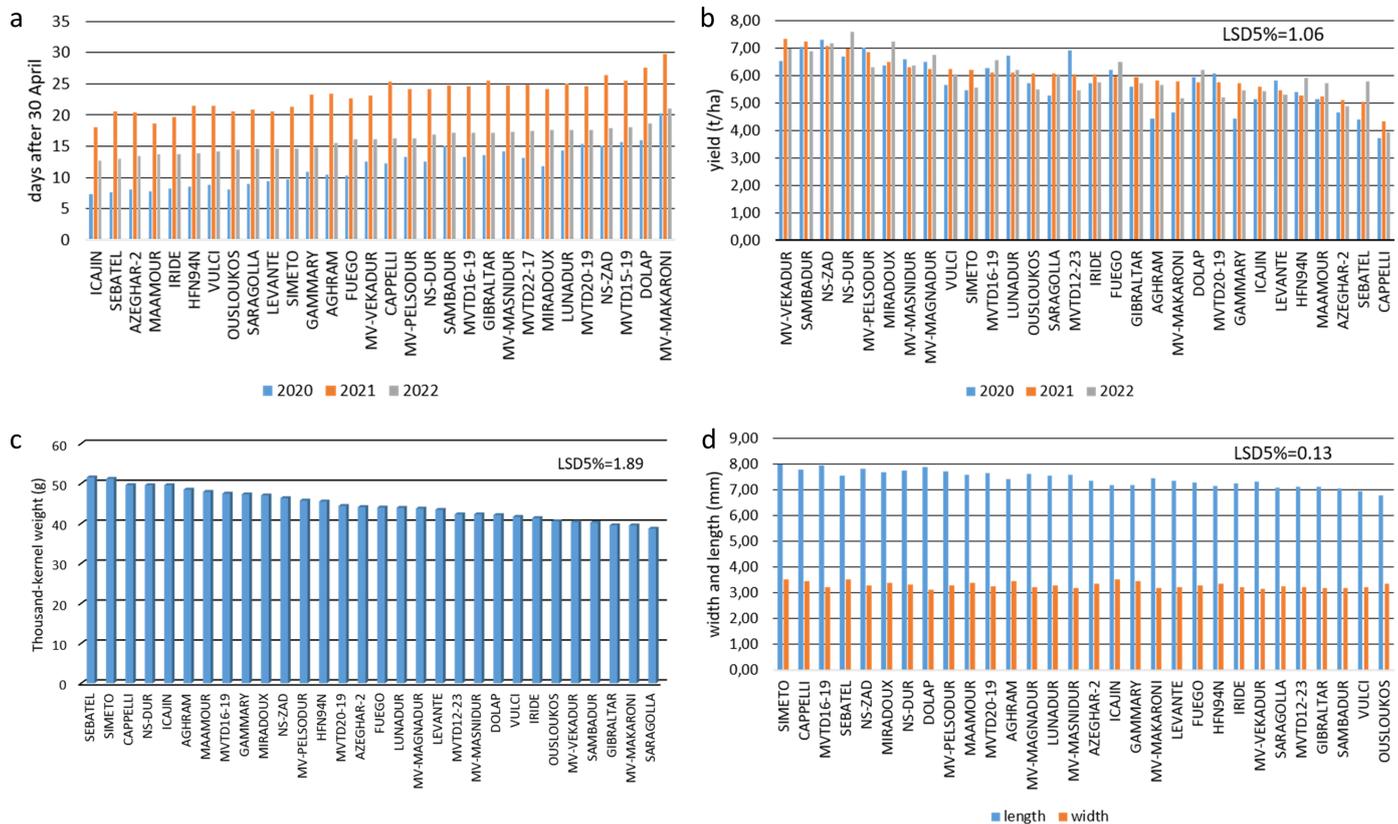
Figure 2 Mean performance of the durum wheat genotypes tested in Martonvásár, 2020-2022: **a** heading date; **b** grain yield; **c** thousand grain weight; **d** grain length and width.

average of the respective experiment (Fig. 2b). TW ranged from 78 to 81 kg hL^{-1} on average (Fig. 3), TKW varied between 42 and 48 g considering the average of the three field trials (Fig. 2c).

Varieties 'Simeto' and 'Cappelli' had significantly longer and wider grains compared to the average of the field trials in all three years (Fig. 2d). A significant and strong correlation was observed between the TKW and grain width, which can be helpful for the breeders in the selection of lines based on grains. Among the naturally occurring pathogens, powdery mildew has appeared every year and in every field. In the wet year 2021 in addition to powdery mildew, leaf spot symptoms and traces of leaf rust and stem rust were also observed.

Keywords

conventional agriculture · grain characteristics · low-input · organic farming · *Triticum durum*
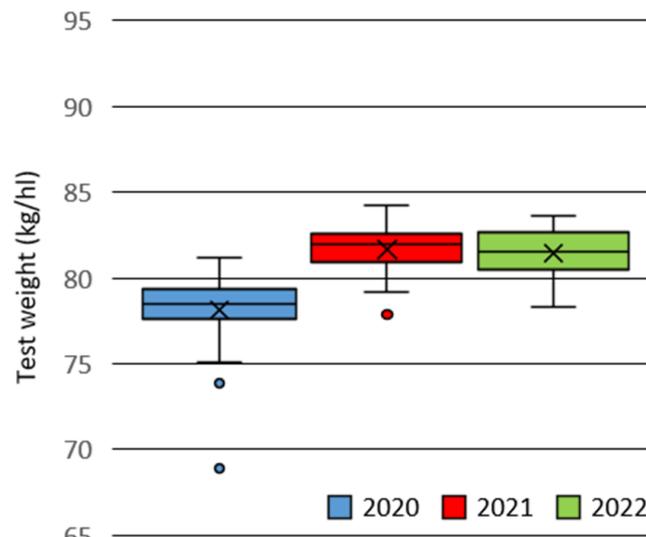
Figure 3 Variability in test weight of the durum wheat genotypes tested in Martonvásár, 2020-2022.

Further reading

Cséplő M, Puskás K, Vida G, Mészáros K, Uhrin A, *et al*. (2024) Performance of a durum wheat diversity panel under different management systems. Cereal Res Commun, in press. DOI: 10.1007/s42976-024-00517-3

Muellner A, Mascher F, Schneider D, Ittu G, Toncea, *et al*. (2014) Refining breeding methods for organic and low-input agriculture: Analysis of an international winter wheat ring test. Euphytica 199: 81-95. DOI: 10.1007/s10681-014-1169-2

Take home organic: Outcomes from the ECOBREED work package 2 (Wheat)

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Abstract

Two diversity panels of winter wheat (*Triticum aestivum*) were established and evaluated for their variability in many phenotypic traits in multi-environment organic field trials (2019-2022) across seven European countries. Genotype by environment interaction was analysed in detail for grain yield, grain protein content and protein yield. Genotypes with high absolute performance across environments were identified as well as genotypes with a high stability of performance. Moreover, genotypes with a high grain protein deviation, *i.e.* trade-off between grain yield and grain protein content, were identified. In another task, the mycorrhiza compatibility of winter wheat genotypes were determined in both greenhouse pot experiments and field trials. These experiments revealed somewhat contradictory results: while pot experiments showed a significant effect of genotypes when inoculated with a specific strain of arbuscular mycorrhizal fungi (AMF), natural infection under field conditions did not show any differences between the tested genotypes. Another task was devoted to weed competitiveness. The used *in vitro* bio-assays revealed that different wheat genotypes had different effects on the germination of two weed species, *i.e. Lolium rigidum* and *Portulaca oleracea*. However, the germination inhibition was only partly accompanied with a higher concentration of secondary metabolites in shoots, roots or the rhizosphere. Allelopathic effects were found to be very complex and not attributable to the accumulation and/or exudation of a single specific compound. Hence, the role of allelopathy in weed competitiveness of wheat besides traits like juvenile growth and early ground cover, plant height, leaf inclination, etc. remains unclear.

Keywords

Allelopathy · breeding · genotype by environment interaction · grain protein content · grain protein deviation · grain yield · molecular marker · mycorrhiza · organic farming · *Triticum aestivum* · weed competitiveness

Introduction

The Horizon 2020 project ECOBREED (ecobreed.eu) aimed to improve the availability of varieties suitable for organic and low-input production of wheat, potato, soybean and buckwheat. Different diversity panels were established in all four crops and tested

in organic multi-environement trials (MET) for agronomic and quality traits. Additionally, other tasks were devoted to (i) the evaluation of mycorrhizal compatibility, (ii) the evaluation of allelopathic effects and weed competitiveness, (iii) the development and/or use of marker-assisted selection for disease resistance and quality traits, and (iv) the development of new germplasm suitable for organic farming. Here we report selected results for common wheat.

Multi-environment organic trials

Two diversity panels of common winter wheat were established considering results from organic variety tests and trial networks, other projects, as well as experience from breeders and farmers. The EARLY nursery included in total 80 genotypes from Austria, Croatia, France, Hungary, Romania, Serbia, Slovakia, and Slovenia. The LATE nursery included 60 genotypes from Austria, Czechia, Germany, and Switzerland. Varieties developed in organic breeding programmes and/or released after organic VCU test were included only in the LATE nursery. Four varieties, *i.e.* 'Arnold', 'Aurelius', 'Capo' and 'Ehogold' were included in both nurseries. Both diversity panels were tested across three years (2020-2022), the EARLY in five countries (HU, RO, RS, SI, SK), and the LATE in three countries (CZ, DE, SK). The final statistical analysis of the MET was done on eight and eleven environments (location×year) for the LATE and EARLY nursery, respectively, as some trials had to be discarded due to *e.g.* damage by hail and/or common bunt (*Tilletia* spp.) infestation.

First, spatial trends were modelled for each individual field trial by mixed model analyses using first-order autoregressive (AR1) functions or other spatial covariance structures (Gilmour *et al.*, 1997). Efficiency of the best spatial model relative to a randomized complete block design was tested (Yang *et al.*, 2004) and best linear unbiased estimators (BLUEs) for genotypes were retrieved from

the best models for the following analysis across environments. Analysis across environments (location×year), was done with genotypes as fixed and environments as random effects. Stability analysis and dissection of the genotype by environment interaction was done by considering univariate and multivariate statistics following both the static and dynamic concept of stability (Becker & Léon, 1988). All statistical analyses were carried out with SAS 9.4 (SAS Institute, Inc., Cary, NC) and Genstat 23rd ed. (VSNi, Hemel Hempstead, UK) software.

Correlation between stability statistics

The correlation between the individual stability statistics for the two diversity panels is displayed for grain yield in Fig. 1. It is obvious that mean rank R_i, site highest yield SHY and superiority index P_i are highly correlated with mean yield ($BLUP$). Due to opposite scaling some of these correlations are negative. Furthermore, high correlations were found between environmental variance s^2_{xi} and the regression coefficient b_i. As both $b_i = 0$ and $s^2_{xi} = 0$ identify a stable genotype according to the static concept the high correlation between these two statistics is consistent in view of their biometrical nature. The same is true for the high correlation between stability statistics based on deviations, *i.e.* the ecovalence W_i, the deviation mean square of regression s^2_{di} and the G×E sum of squares due to to imperfect correlation $SSIC_i$ The two non-parametric measures mean rank difference S_i^1 and variance of ranks S_i^4 show high correlations only to each other and only low to medium correlation to W_i and s^2_{di}. Similarly, G×E due to heterogenous variances $SSHVi$ is more a stand-alone measure with only moderate correlation to W_i.

In Table 1, MET results are presented for SHY as relative measure of performance and for W_i, s^2_{di} and $SSIC_i$ as measures of stability. SHY was introduced by Jensen (1976) as 'floating check' for a two-way G×E matrix whereby the absolute values of performance of
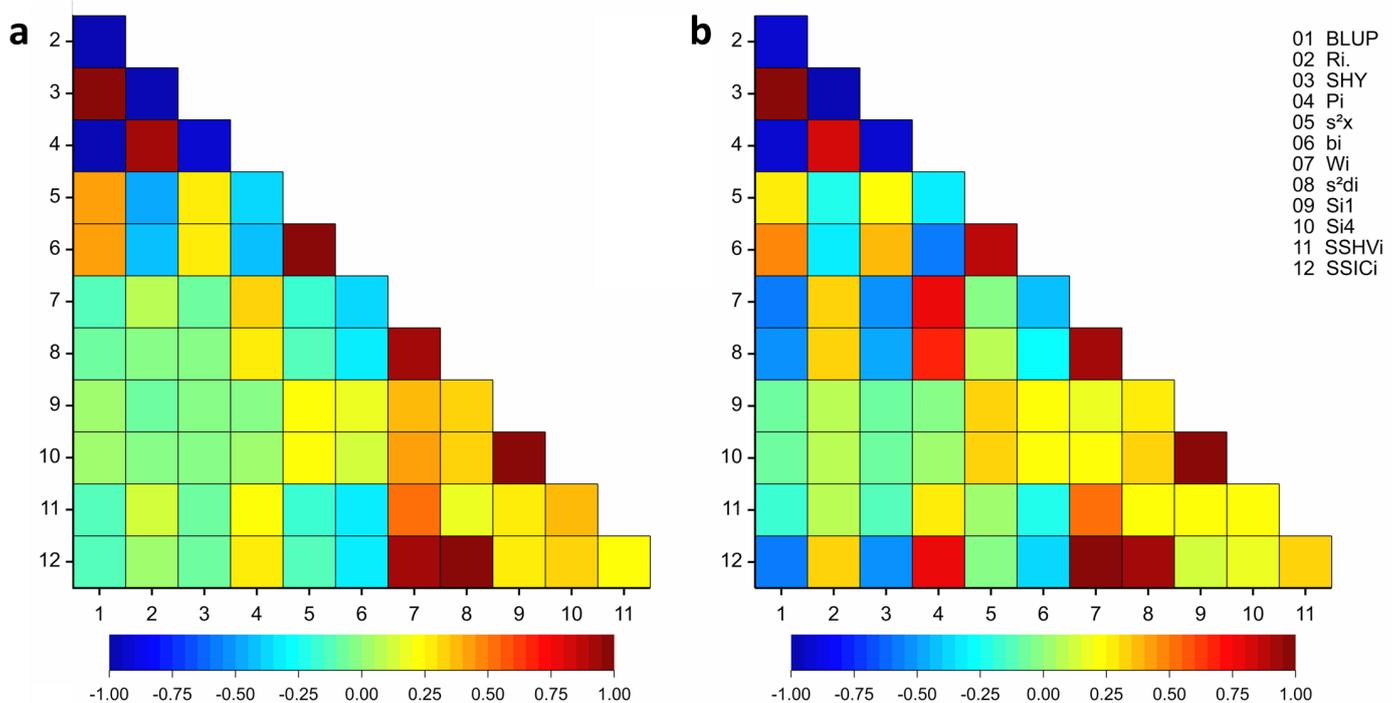
Figure 1 Correlation matrix of stability statistics for grain yield of **a** the EARLY and **b** the LATE common wheat diversity panel. *BLUP*, best linear unbiased predictor; R_i, mean rank; *SHY*, site highest yield floating check; P_i, superiority index; s^2_{xi}, environmental variance; b_i, regression coefficient; W_i, ecovalence; s^2_{di}, deviation mean squares; S_i^1, mean rank difference; S_i^4, variance of ranks; *SSHV*_i, heterogeneous variance; $SSIC_i$, imperfect correlation.

each environment are transformed into relative values with the highest performance having always a value of 100. This procedure allows an automatic adjustment to the fertility level of each environment. It is obvious from Table 1 that the yield potential of the LATE nursery is slightly higher compared to the EARLY nursery. While in the EARLY nursery none of the stable genotypes shows grain yields above average, seven varieties of the LATE nursery are among the most stable and above average performing (*i.e.* 'Penelope', 'Moschus', 'Rübezahl', 'Genius', 'Graziaro', 'Curier'). Varieties released after organic VCU test are especially present in the most stable performing genotypes, while the highest yielding 'organic' variety 'Wendelin' has an average stability.

Protein content and grain protein deviation

Grain protein content (GPC) was generally low in both diversity panels and ranged in average from 8.14% (RO21) to 10.36% (SI20) for the EARLY nursery, and from 8.27% (SK20) to 11.46% (CZ21) for the LATE nursery. This demonstrates clearly that realisation of a GPC as high as possible to fulfill requirements by traders and the baking industry is a major challenge under organic management. Low yielding landraces, *e.g.* 'Bánkúti 1201', 'Radošínska Karola' and 'Stupicka Bastard', showed generally high GPC values, how-

ever, similar high GPC were also realized by modern varieties, *e.g.* 'FDL Amurg', 'Mv Elit CCP', 'Izalco CS', 'Mv Magdaléna' and 'PS Dobromila' in the EARLY nursery. In the LATE nursery, the majority of varieties from organic breeding programmes were the top performing varieties, *i.e.* 'Prim', 'Tengri', 'Philaro', 'Pizza', 'Wiwa', 'Butaro', 'Wendelin', 'Wital', 'Tobias', 'Thomaro', 'Poesie', 'Arminius', and 'Jularo'. 'Arnold' was the only conventionally bred variety with similar high GPC. The sum of standardised residuals from the regression of grain yield on protein content as indicator of grain protein deviation (GPD) identified 'Mv Kolompos', 'Mv Káplár', 'Mv Kepe', 'Mv Karéj' and 'CCB Ingenio' in the EARLY, and 'Wendelin', 'Turandot', 'Elixer' and 'Arnold' in the LATE nursery as those varieties with the greatest trade-off from the negative correlation.

**Plant-microbe interactions**

Pot and field experiments were carried out to (i) test the capability of wheat verieties to form a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), (ii) investigate the natural AMF flora in organic and conventional fields, and (iii) to test the effect of seed priming with biostimulants. Increased AMF colonisation would be beneficial as it has the potential to reduce phosphorus fertiliser

Table 1 Performance of European winter wheat varieties from the ECOBREED diversity panels EARLY (left) and LATE (right). Displayed varieties include genotypes with *SHY* higher than the mean performance and the most stable genotypes (grey background colour) according to ecovalence. Varieties released after organic VCU test are printed in bold. *SHY*, site highest yield (Jensen, 1976); W_i, ecovalence (Wricke, 1962); s^2_{di}, mean square deviation (Eberhart & Russell, 1966); *SSIC_i*, imperfect correlation variance (Muir *et al.*, 1992); GY, grain yield (kg ha^{-1}).

Genotype	SHY	W_i	s^2d_i	$SSIC_i$	Genotype	SHY	W_i	s^2d_i	$SSIC_i$
Mv Káplár	88.7	132.4	25.4	18.9	Turandot	93.5	72.2	11.1	14.9
Mv Kepe	86.5	431.0	28.3	21.0	Elixer	93.1	174.9	34.3	21.1
Mv Kolompos	85.1	433.2	47.0	27.8	Liseta	91.4	213.1	30.4	23.4
Mv Menrot	83.6	370.3	35.1	23.4	SG-S1004-18	88.1	74.5	4.1	11.2
Mv Karéj	83.1	455.5	46.6	28.2	Viki	86.8	133.3	13.3	16.4
Sofru	82.6	480.4	30.2	20.9	SG-S269-09	86.7	112.1	16.2	15.7
CCB Ingenio	81.7	316.4	22.1	17.1	Asory	86.3	146.7	22.2	17.6
FDL Abund	79.8	137.6	10.0	13.3	Ibarra	86.0	237.3	37.6	23.8
Illico	80.5	134.6	15.6	14.4	Argument	85.6	193.5	14.0	16.8
Mv Uncia	78.8	133.8	13.7	14.3	Sultan	84.4	151.7	20.9	18.3
Nikol	79.9	350.4	26.9	19.5	KWS Milaneco	84.0	451.2	26.7	21.2
Unitar	77.7	428.3	19.9	17.2	**Wendelin**	83.1	181.6	27.1	21.8
Mv Lucilla	77.4	401.5	17.9	15.9	KM78-18	83.1	108.8	10.6	13.7
Solehio	75.8	437.1	25.4	18.8	**Naturastar**	82.9	210.6	18.3	19.9
Ursita	76.6	453.9	13.5	14.9	Annie	82.2	208.1	29.6	22.9
NS Mila	77.7	313.4	22.2	17.7	Ehogold	81.8	239.6	34.5	25.5
NS 40S	75.5	375.3	16.1	15.3	Illusion	81.5	96.7	16.9	16.1
Simnic 60	75.4	422.4	15.1	15.2	Capo	81.1	172.2	12.2	17.2
NS Obala	74.5	401.0	15.6	15.4	Dagmar	80.8	151.6	23.0	19.9
NS Ilina	74.8	416.0	13.1	14.2	**Purino**	80.3	113.8	21.0	17.5
Adelina	69.9	27.6	2.4	9.3	**Blickfang**	78.4	17.7	2.5	11.5
Savinja	73.5	34.1	3.6	9.5	**Rübezahl**	82.2	33.0	0.3	9.9
PS Dobromila	66.8	59.0	5.5	10.3	**Poesie**	71.7	41.1	3.3	11.5
Vulkan	74.3	64.4	7.3	11.1	**Jularo**	73.7	41.3	6.4	12.2
Bertold	68.3	68.7	7.4	11.6	Genius	81.9	46.4	7.9	13.3
Juno	71.4	69.9	7.4	11.6	**Arminius**	76.5	50.7	9.7	13.6
Mv Suba	66.3	71.9	7.4	11.7	**Graziaro**	80.8	54.4	9.0	13.8
Edelmann	69.4	73.4	7.6	11.1	**Pizza**	72.2	56.3	7.5	13.7
11248G2-1	62.8	78.5	8.0	11.6	Penelope	85.0	56.9	8.3	14.0
PS Kvalitas	68.6	78.5	8.8	12.0	**Curier**	78.8	57.6	7.1	13.7
Zvezda	69.9	80.2	8.2	12.0	Moschus	84.2	69.4	6.6	13.5
Mv Mente	71.2	83.3	5.7	10.3	**Aristaro**	76.2	69.8	13.5	14.8
Mean SHY	74.6	236.0	19.8	16.9	Mean SHY	78.4	198.5	26.2	20.87
Mean GY (100%)	5407				Mean GY (100%)	7096			

inputs and the environmental consequences of increased phosphorus supply to water systems (Campos *et al.*, 2018).

The pot experiments were carried out with 40 genotypes selected mainly from the EARLY and LATE panels. The growth medium was treated with *Funneliformis mosseae* strain BEG12. Plants were harvested after 8 weeks of growth, the roots were washed and root and shoot fresh and dry weight was measured. Analysis of AMF colonisation was carried out by staining roots (Vierheilig *et al.*, 1998). AMF root colonisation was determined under a light microscope (Fig. 2) on 30 root pieces per sample for intraradical hyphal (*H%*), vesicle (*V%*) as well as arbuscule abundance (*A%*). Out of these data, parameters such as mycorrhizal frequency (*F%*) and intensity of mycorrhizal colonisation (*M%*) in the root system can be calculated (Trouvelot *et al.*, 1986).
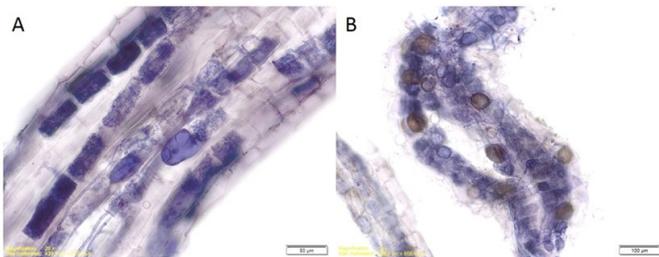

Figure 2 Arbuscular mycorrhizal structures in wheat roots from field samples stained with ink and vinegar: **a** cv. 'Aurelius'; **b** cv. 'Arminius'.

All 40 wheat genotypes were colonised by *F. mosseae* during the growth period. The genotypes did not differ significantly with regard to *F%*. Considering *M%*, 'Spontan' and 'Bauermeister' of the first screening and 'NS_Mila' from the second screening showed the highest intensities (20-26%), whereas 'Ehogold' and Xt88.5R had the lowest intensities of 2% and 1%, respectively. At the same time, 'Spontan' and 'Bauermeister' also showed higher *Vr%* compared to the other genotypes (Fig. 3a). Similar dynamics were observed in the second screening (Fig. 3b). This also indicates that AMF are already entering the vesicle stage important for storage and survival without a living host plant.

In order to screen for native AMF colonisation in the field, an organic field trial with 6 wheat varieties was established. Root samples were taken at BBCH 55 (middle of heading). In each plot, 4 root samples were taken at a depth of 10 cm. Roots were washed and root fractions were split into two parts. Contrary to the pot experiment, all genotypes showed similarly high *F%* in the field. Only 'Bauermeister' with very high *F%* in the greenhouse experiment showed slightly lower values in the field trial. In all genotypes, AMF had formed all three structures such as hyphae, arbuscules and vesicles to nearly equal amounts. With regard to AMF development, the relative number of vesicles was much higher compared to the relative number of arbuscules. Overall, it can be concluded that the selected field plots had a high prevalence of native AMF and that all genotypes formed readily arbuscular mycorrhiza with the local AMF strains.

Two commercial biostimulant products (*i.e.* RhizoVital® 42F - *Bacillus velezensis* FZB42 and T-Gro Easy-Flow® - *Trichoderma asperellum*) were used for seed priming of cvs. 'Aristaro' and 'Capo'. The treated seeds were sown together with an untreated control mid-October 2021 and 2022 on an organic field in Raasdorf, Austria.
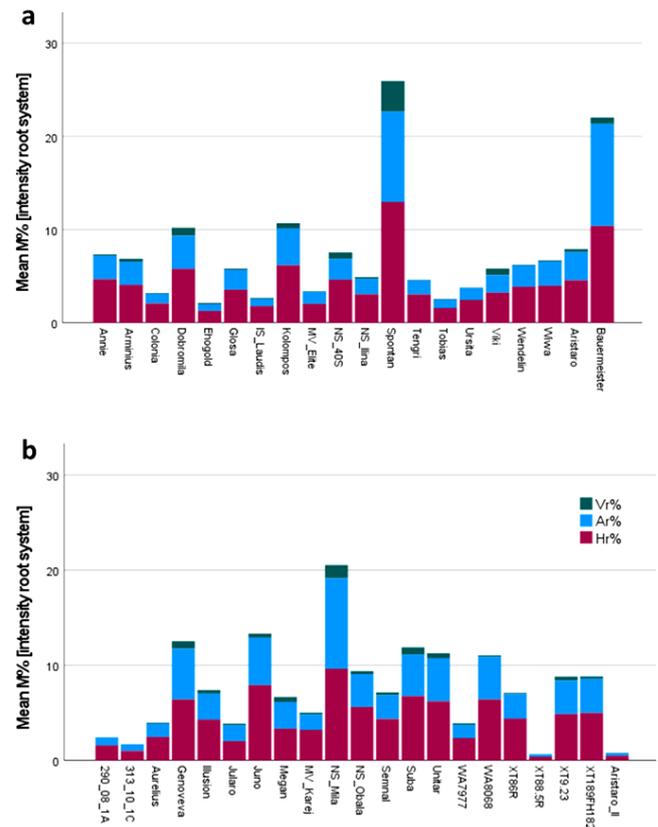
Figure 3 Mean intensities of AMF in the root system (*M%*) of wheat genotypes presented as relative amounts of vesicles (*Vr%*), arbuscules (*Ar%*) and hyphae (*Hr%*): **a** subset 1; **b** subset 2. Inoculation of the growth medium was done with *F. mosseae* BEG12.

Plot size was 150 m² in the first, and 300 m² in the second year. Combine harvest was done on 25 m² subplots and the following traits were measured: grain yield, test and thousand grain weight, seed plumpness >2.5 mm and GPC. Analysis of variance revealed only a significant effect of the genotype but no significance between the different biostimulants. Although it seemed that the treated plots had a faster and better emergence after sowing, finally no significant effect was obvious for any trait and treatment at harvest. A field experiment by Nguyen *et al.* (2019) showed also no effect for FZB42 treated seeds on wheat quality (measured as Zeleny sedimentation volume) and a significant increase of grain yield only in one of the two experimental years and only in case of very low nitrogen availability. The authors conclude that N fertilizer level, time of application of the plant-growth promoting rhizobacteria (PGPR) and temperature play a major role for the plant growth-promoting capacity of the applied PGPR strains for winter wheat under temperate field conditions. Similarly, Illescas *et al.* (2022) demonstrated a positive effect of *T. asperellum* on wheat grain yield under water stress conditions. In summary, further research is needed to unravel the interaction between wheat genotypes and PGPR under different environments and management conditions. Moreover, effectiveness of PGPR as biostimulants may be influenced also by the way of application, *e.g.* seed priming, soil irrigation or foliar spray.

Plant-plant interactions

Allelopathy refers to plant-plant interaction mediated mostly by secondary metabolites ('allelochemicals') that act directly or indirectly phytotoxic to neighbouring plants. Allelochemicals can be

released by air (volatilization), the soil (plant residues and root exudates), leaching or transformed by microorganisms in the soils to create other molecules (Kobayashi, 2004).

The allelopathic potential of 30 wheat varieties against two very aggressive and herbicide-resistant weeds in Europe, the dicot common purslane (*Portulaca oleracea*) and the monocot annual ryegrass (*Lolium rigidum*), was evaluated after *in vitro* co-cultivation of the wheat varieties with the weeds (Wu *et al*., 2000). First, germination rate (*G%*), plant weight (*PW*), shoot (*SL*) and root length (*RL*), and plant height of the weeds were measured to obtain information on the inhibition or stimulation of weed growth (Fig. 4), the ability of weeds to colonise the space through germination and development (*SI*C: shoot invasive capacity; *RIC*: root invasive capacity) and how weed plants can develop in the next generation (*SVI*: seedling vigour index). *SL* and *RL*, and *PW* were also measured for the wheat plants. Second, allelochemicals from the shoots and roots, as well as root exudates from wheat and weeds were quantified to evaluate the accumulation or release of these compounds by the different wheat varieties in presence of the weeds.
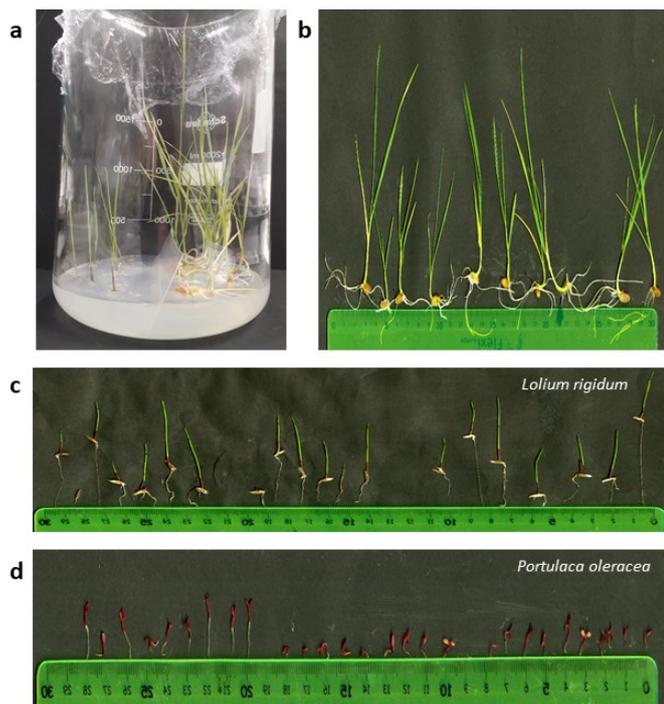
In general, the wheat varieties showed a greater ability to control annual ryegrass than common purslane. The *in vitro* bioassay showed that all genotypes except 'Glosa' inhibited at least one of the tested parameters in annual ryegrass. Cvs. 'Ludwig', 'Maurizio' and 'NS Azra' inhibited all seven parameters (*G%*, *PW*, *SL*, *RL*, *SIC*,

Figure 4 *In vitro* bioassay for allelopathy: **a** co-cultivation of annual ryegrass and wheat in the equal-compartment-agar method. Determination of shoot and root traits of **b** germinated wheat; **c** annual ryegrass; and **d** common purslane.

RIC and *SVI*); six varieties ('Capo', 'Midas', 'NS 40S', 'Proteinka', 'PS Dobromila' and 'Tobias') inhibited 6 out of 7 parameters, and another three varieties ('Bezostaya 1', 'Ehogold' and 'IS Laudis') inhibited 5 out of 7 parameters. 'Alessio', 'Arminius' and 'Element' stimulated *RL* without altering *PW* or *SL*. The most affected parameter in annual ryegrass was *RIC* which was inhibited by 22 out of the 30 wheat varieties. Most of the varieties (21 out of 30)

induced inhibition of germination, while 'Element' stimulated germination. In summary, the greatest allelochemical effect against annual ryegrass was exhibited by 'NS Azra', followed by 'Ludwig', 'Maurizio', 'PS Dobromila', 'Midas' and 'Tobias'.

In contrast, the bioassay with common purslane showed that none of the tested varieties inhibited more than four measured parameters; just 'Capo' and 'Glosa' were able to inhibit four parameters (G%, PW, RL, RIC and PW, RL, RIC, SVI, respectively), while 10 varieties ('Adesso', 'Annie', 'Capo', 'Ludwig', 'Midas', 'NS Azra', 'Spontan', 'Tobias', 'Ubicus' and 'Ursita') inhibited three parameters.

The chemical analysis also showed different profiles of accumulation and/or exudation of allelochemicals in the different wheat genotypes which could be related to their ability to compete with the weeds. Different allelochemicals (benzoxazinoids and polyphenols) were detected in the shoots, roots and agar (root exudates) of the wheat genotypes when grown alone as control. However, the allelochemicals profile changed in the presence of weeds depending on the variety studied. This was especially true for the benzoxazinoids BOA, DIBOA, HBOA, MBOA, DIMBOA and HMBOA. Regarding phenolic compounds, a similar pattern of accumulation or exudation was observed for the different genotypes and co-cultivated target weed.

The studies revealed that different wheat genotypes have different abilities to manage neighbouring weeds by allelopathy and that the same genotype can behave differently depending on the weed species. The screening of wheat varieties for allelopathic effects is crucial to move forward in organic weed management as allelopathy can support other control measures of weed management, *e.g.* harrowing or plant traits such as plant height. In particular the latter control measures might have limitations, *e.g.* harrowing may not be possible in the right time due to weather conditions or may damage the crop stands due to inappropriate usage or time; too tall plant height may cause severe lodging and, therefore, grain yield losses. In our research, a correlation between root exudation and weed inhibition was found. However, not all varieties have allelopathic potential. Summarizing, the most promising varieties to control annual ryegrass according to the *in vitro* bioassay are 'NS Azra', 'Ludwig', 'Maurizio', 'PS Dobromila', 'Midas', and 'Tobias', while 'Capo', 'Glosa', 'Adesso', 'Annie', 'Ludwig', 'Midas', and 'NS Azra' were the most phytotoxic against common purslane. We conclude that different plant genotypes have different capacities to synthesise and exude specialised metabolites, resulting in a wide range of allelopathic or competitive effects on weed germination and seedling growth, also depending on the prevailing weed species. It is essential to understand the mode of action of allelochemicals as well as the role of other traits, *e.g.* early vigour, plant height, growth habit, etc., to identify and select wheat varieties suited to organic and low-input farming systems. Considering all traits determined in our studies and the field phenotyping, the most promising varieties for weed control of the tested diversity panel are 'Ludwig' and 'Tobias'.

Marker-assisted selection

Molecular marker protocols were applied to screen the EARLY and LATE nursery for the presence of genes related to disease resistance, adaptation and agronomic traits. A wheat-rye chromo-

some translocation and thereby the presence of the *Sr31/Lr26/Yr9/Pm8* gene complex was observed in 11 genotypes which represents a frequency of 8.1%. While the 1BL.1RS translocation was present in various breeding programmes, the 1AL.1RS translocation was found only in the breeding programmes of Secobra, DE, and NARDI, RO. Most widely exploited in the two diversity panels is the *Sr38/Lr37/Yr17* gene complex from *T. ventricosum* which is present in 27 (35.5%) and 29 (48.3%) genotypes of the EARLY and LATE nursery, respectively, followed by *Lr1* (28.9% and 31.7%). *Lr10, Lr34* and *Lr46* are present in about 20-30% of the EARLY panel, while these genes were rarely present in the LATE nursery. Only in cvs. 'Graziaro', 'Vulkan' and the populations 'Brandex' and 'Liocharls' (all *Sr15*) other *Sr* genes than *Sr31* or *Sr38* are present. With respect to yellow rust, both diversity panels lack *Yr* genes broadly effective against the current races of the pathogen, *e.g.* *Yr15*. The latter resistance gene was verified for other European wheat germplasm, *e.g.* 'Blackstone', 'Champion', 'Kapitol', 'KWS Dawsum', 'KWS Ultimatum', 'LG Beowulf', 'Shrek' and 'WPB Match'. Furthermore, molecular markers were established and verified for resistance QTL against common bunt (*Tilletia* spp.) (Lunzer *et al.*, 2023a,b). Breeding lines with improved bunt resistance developed by MAS were distributed to commercial breeders and are currently under selection for further traits.

Five LATE genotypes carry the *Ppd-D1a* allele for photoperiod in-sensitivity, whereas in the EARLY panel 62.3% of the genotypes carry this allele. The dwarfing allele *Rht-B1b* is present in 43.8% of the EARLY nursery; this dwarfing allele is especially prevalent in modern Hungarian and Romanian varieties and breeding lines, while *Rht-D1b* is present only in 'Mv Karizma' and 'Anapurna'. Contrary, in the LATE nursery *Rht-B1b* is present only in 'Asory', *Rht-D1b* has a slightly higher frequency, being present in four varieties ('Asory', 'Curier', 'Moschus' and 'Rübezahl').

Acknowledgements

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Towards innovative variety testing - The Klimafit and ADAPT projects

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Abstract

Value for cultivation and use (VCU) testing is part of the official variety registration process. VCU testing relies on established protocols and evaluates the characteristics of candidate varieties in comparison with already registered varieties. Climate change calls for varieties that cope with its impacts like severe spells of heat and drought, or heavy precipitation events. In the research projects Klimafit I & II and ADAPT, wheat, soybean, and potato trials with and without irrigation were conducted. The Klimafit I & II projects are joint national research projects led by Saatgut Austria in cooperation with eight breeding companies and the AGES. The overarching goal is to develop new varieties in all major crop groups for Austria, with a particular focus on drought and heat tolerance. The ADAPT project brings together a European consortium of 18 partners to develop new strategies to make potatoes fit for the challenging growth conditions of the future.

In these projects, the AGES, as the institution responsible for variety testing in Austria, evaluates the use of innovative technologies to assess abiotic stress tolerance of different varieties during VCU trials, with special focus on drought and heat stress. In addition to standard parameters according to the established VCU protocols, drone and environmental data were collected and are being evaluated for possible integration into VCU testing. The same set of varieties of soybean, wheat, and potato were grown under irrigated and non-irrigated conditions at the AGES field trial site 2286 Fuchsenbigl in 2022 and 2023.

In addition to standard VCU assessments, we evaluated the potential of integrating data collected by two drone flights at specific timepoints and environmental sensors throughout the growing season. The drone collected stress indices in plant growth based on RGB and multispectral imaging, like NDVI (Normalized Difference Vegetation Index), WDVI (Weighted Difference Vegetation Index), and CIRED (Chlorophyll Index based on red and near-infrared). Furthermore, the following parameters were considered: the progress and development of vegetation coverage, and the continuous monitoring of air temperature and humidity as well as soil temperature and moisture in 5 cm and 20 cm soil depth. The data collected in the field are in part complemented by RNA- and metabolomic analyses performed on leaf samples collected in the trials.

The integration of such parameters into VCU testing requires specific considerations, in particular concerning data reliability, reproducibility, and comparability over several trial seasons. Using new technologies has a great potential for resource efficiency by po-

Figure 1 Different technological approaches to identify relevant characteristics associated with responses of potato to abiotic stress factors in the Austrian field trials: (A) drone flights, (B) environmental sensors, (C) evaluation of tuberization, (D) sprinkler irrigation, (E) leaf sampling for RNA and metabolomic analyses.

Ribarits A, Bomers S, Von Gehren P, Kirchmaier S, Söllinger J (2024) Towards innovative variety testing - The Klimafit and ADAPT projects. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 74. Jahrestagung 2023, 20-22 November, Raumberg-Gumpenstein, pp 59-60. BOKU University, Vienna, Austria. ISBN-13: 978-3-900397-13-5

tentially reducing time and effort spent on VCU trials as well as advancing VCU protocols. Big amounts of data can be collected in short time and allow for objective, data-based phenotyping of varieties. Our experiments also showed that the spatial resolution of drone flights imaging is well suited for VCU plot sizes, and that differences in abiotic stress tolerance of varieties can be visualized. However, especially the high amount of expertise required for drone flights (including legal ramifications) and pre- and post-flight data processing must be mentioned. Additionally, the timing and the ideal conditions for the drone flights to detect abiotic stress tolerance needs further consideration. Finally, the reliability of stress indices under varying climatic conditions, especially important for a comparative assessment like VCU trials, has not been fully determined yet.

Keywords

abiotic stress tolerance · drone flights · environmental sensors · field trials · multispectral imaging · value for cultivation and use (VCU)

European potato farmers need adapted varieties to meet a changing climate – Survey results from the research project ADAPT

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Abstract

Extreme weather events associated with a changing climate - heat waves, droughts, heavy precipitation - as well as a changing spectrum of plant diseases and increased occurrence of plant pests could severely threaten crop production in Europe (Madsen *et al.,* 2014; Haverkort *et al.*, 2008). The abiotic stress-sensitive potato (*Solanum tuberosum L.*) prefers a temperate climate, and high temperatures during the growing season impair tuber development leading to significantly decreased yield. In changing climatic conditions, European potato farmers will have to take up adaptation strategies to retain high yield and high quality. Commonly, adaptation strategies aim at reducing the negative impact of drought, and include, among others, adjusting of planting dates to more favorable conditions, improving water management, and planting better-adapted varieties (Macholdt & Honermeier, 2018; von Gehren *et al.*, 2023). For effective climate change adaptation, farmers need to be aware of the risks associated with climate change and be able to take up (multiple) adaptation measures. Farmer surveys are a useful tool to assess the adaptive capacity of farmers and can shed light on which adaptation policies might have the biggest impact (Simelton *et al.*, 2013).

In the frame of the Horizon 2020 project ADAPT (Accelerated Development of multiple-stress tolerant Potato) a survey among European potato farmers assessed their perception of climatic changes and how this impacts potato production. Furthermore, it was investigated which adaptation measures farmers are willing to implement. The survey was accessible online over a period of nearly five months, from December 2020 until April 2021. In the end, 553 potato farmers from 22 different European countries participated in our survey, with most respondents being from Austria, the Netherlands, Germany, France, and Switzerland, followed by Slovenia, Belgium, Poland and Spain. Most survey respondents were experienced potato farmers who had been growing potatoes for more than five years.

In our survey, 89.2% of farmers responded that changes in climatic conditions had been affecting their potato production in the last ten years. Of those, the majority of respondents had noticed the effects of drought and heat (87.4% and 82.6%, respectively), followed by pests and pathogens induced by climatic conditions (53.3%) and heavy precipitation events (42.6%), among others. Heat and drought were also considered the greatest threat to maintain potato production in the future.

When asked about their preferred adaptation strategy, 73.2% of farmers favored planting an adapted variety, followed by irrigation (44.8%), change of planting / harvesting date (44.7%), adopting different tillage practices (43.9%) and changes in crop rotation (32.0%). When specifically asked if the impact of climatic changes on potato production could be reduced by the choice of an adapted variety, 74.0% of survey respondents agreed to this statement. To understand what farmers are looking for in an adapted variety, survey participants were asked to rate how important they consider certain potato characteristics for an adapted variety (Figure 1). Yield stability, heat tolerance, disease resistance, drought tolerance, and yield potential were highly desired traits. Pest resistance as well as dormancy and storability were considered slightly less important.

Our survey results also show that farmers tend to rely on their own experience (81.2%) or that of their peers (57.3%) when choosing their varieties. Less importance is attached to infor-
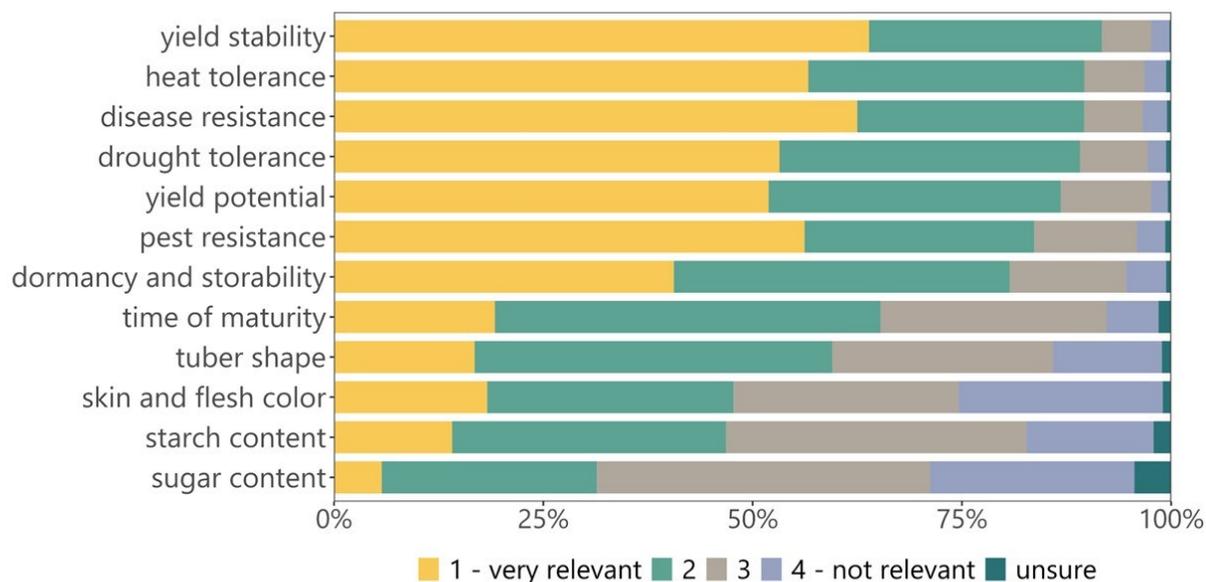
Figure 1 Rating, ranging from 1 = very relevant to 4 = not relevant, given by survey participants (European potato farmers) to the question "How important do you consider these characteristics for the choice of an adapted variety?". Characteristics in the figure are ranked depending on the most replies assigned either value 1 or value 2 at the top.

mation provided by breeding companies (38.2%) or plant variety examination offices (33.5%). Overall, the survey results highlight the challenges European potato farmers are facing in times of climate change and the crucial importance of research and breeding activities that support farmers in effectively mitigating the effects of a changing climate by supplying them with better adapted varieties. More detailed survey results are published in von Gehren *et al*., 2023.

Keywords

Breeding · climate change adaptation · drought stress tolerance · heat stress tolerance · *Solanum tuberosum L.*

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Influence of drought on cocksfoot cultivars on yield and water use efficiency

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Abstract

Growth and productivity of forage crops are influenced by various biotic and abiotic factors, in particular climate change-induced changes in the distribution of precipitation and the more frequent occurrence of droughts (IPCC, 2022). In alpine regions and other less-favourable areas, where the options for crop farming and ley cultivation are restricted, permanent grasslands serve as the predominant form of land use (Buchgraber *et al.*, 2011). Nevertheless, permanent grassland has a significantly greater need for water than cultivated fields, rendering it more susceptible to the impacts of drought conditions. For this reason, on drought-prone sites, the composition of seed mixtures should be shifted toward more drought-tolerant species. In this context, cocksfoot (*Dactylis glomerata*) is of particular importance. Cocksfoot, although typically offering lower forage quality than perennial ryegrass, compensates with robust yield performance during summer and enhanced tolerance to abiotic stress factors, making it a reliable choice in challenging growing conditions (Sanada *et al.*, 2010). While the species is considered to be very drought tolerant, very little is known about varietal differences as a standard survey in field trials is very difficult.

Accordingly, a trial was started in the greenhouse of the HBLFA Raumberg-Gumpenstein to investigate the drought tolerance of five cocksfoot cultivars ('Amba', 'Laban', 'RGT Lovely', 'Prolana' and 'Tandem') of different European geographical origin under controlled conditions. These cultivars were subjected to four irrigation treatments, repeated six times in a randomized order across 120 pots over three growths. The primary aim was to investigate the effects of three field capacity (FC) levels (Treatment 1: 80%; Treatment 2: 60%; Treatment 3: 40%), focusing on yield and water use efficiency. The experimental setup involved sowing plants in standard soil, transplanting them into seed trays, and eventually repotting into Mitscherlich pots filled with quartz sand. These pots had a covered water drain to prevent root penetration and substrate leakage. Initial fertilization was provided and the field capacity was calculated as the difference between the saturated weight and the dry weight of the substrate. The water content of the pots was monitored gravimetrically multiple times per week, adjusting irrigation to maintain the specified weight.

The study revealed that the highest yields across all growth stages and cultivars were observed in Treatment 1 (T1), with a gradual decrease in yields noted in Treatments 2 (T2) and 3 (T3). The first growth consistently produced the highest yield across all cultivars and treatments.

The findings together with results from literature indicate a recurring observation: drought does impact all cultivars but not in a uniform manner (Bristiel *et al.*, 2019). Among the cultivars, 'Prolana' stood out with the highest average yield across all growths and treatments, significantly outperforming 'Tandem' and 'Laban'. Examining the treatments, 'Lovely' and 'Tandem' exhibited impressive yields in all growths under T1, consistently surpassing the treatment average. In T2, 'Prolana' yielded the highest, significantly outperforming 'Laban'. In T3, both 'Prolana' and 'Amba' had higher yields than the treatment average cumulatively, with 'Prolana' exceeding the treatment average in each growth.

In contrast to the results of dry matter production, the results of water use efficiency (WUE) were exactly opposite. The study found a systematic enhancement in WUE from T1 through T2 to T3, consistent across all cultivars except 'Tandem' in the second and 'Amba' in the third regrowth suggesting that as the water availability decreased, the cultivars utilized water more efficiently. This is consistent with the results of Kørup *et al.* (2018) who also investigated the WUE of cocksfoot and other perennial grasses under drought conditions. The highest WUE was observed in the second growth for all cultivars. Throughout the entire experimental duration, 'Tandem' consistently exhibited the highest average WUE among all cultivars and treatments. This superiority in WUE was statistically significant when compared to 'Amba' in the second and 'Prolana' in the third growth. Moreover, Tandem's superiority in WUE was not limited to a specific treatment but was evident across all conditions, highlighting its robustness and efficiency in water utilization under varied irrigation regimes.

The study demonstrated that drought stress significantly impacts the dry matter yield and WUE of cocksfoot cultivars. Variations were observed in yield and WUE across cultivars and irrigation levels, emphasizing the importance of cultivar selection and water management in forage production under varying climatic conditions.
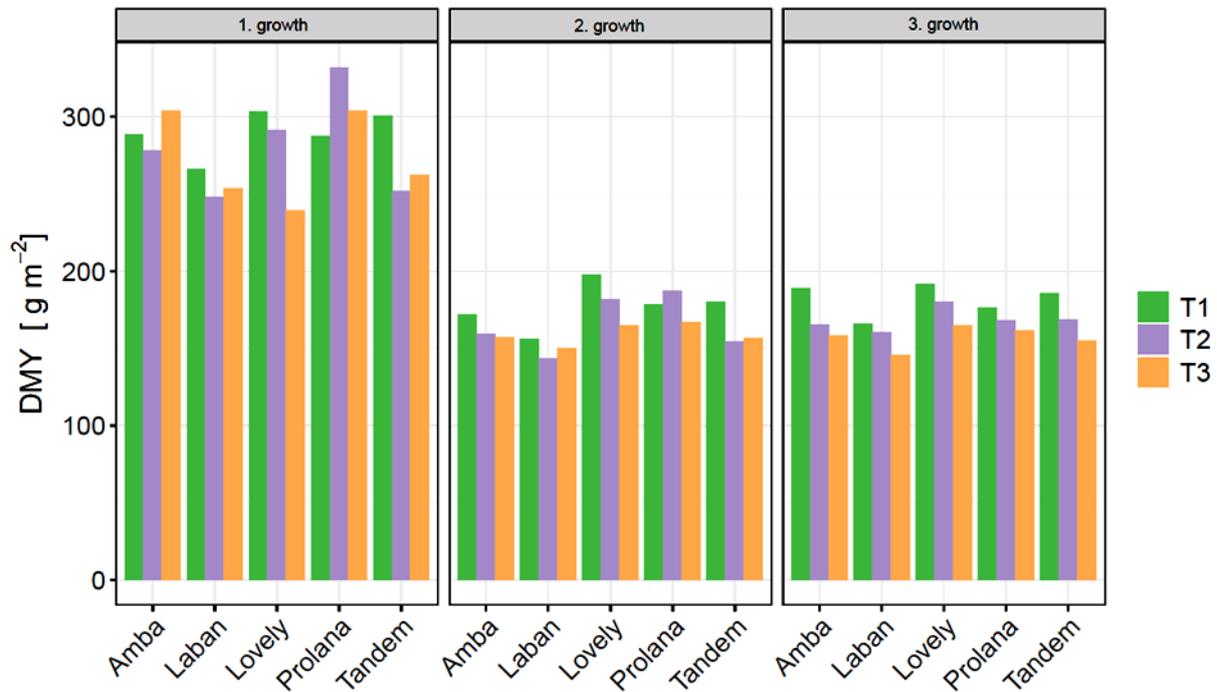
Figure 1 Dry matter yield of cocksfoot cultivars from first to third growth under different field capacity levels (T1=80% FC, T2=60% FC, T3=40% FC).

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