HUNGARIAN UNIVERSITY OF AGRICULTURE AND

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STUDY OF THE FEASIBILITY OF DIGITAL IMAGE PROCESSING IN WHEAT CULTIVATION

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THESIS OF DOCTORAL (PhD) DISSERTATION

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1. BACKGROUND OF THE WORK, AIMS

Agriculture in Hungary plays an important role in the economic and social spheres, as well as in the environmental and nature conservation, therefore it is important to develop sustainable agricultural practices. Industrialised, intensive, material- and energy-intensive agriculture results in homogeneous, monocultural landscape that fail to fulfil their ecological functions, reduce biodiversity, and increase soil and water pollution (Batáry et al. 2011, Sutcliffe et al. 2015, Emmerson et al. 2016). It can also contribute to rural unemployment and accelerate rural outmigration by displacing live labour (White 2012).

Climate change and the increasing frequency of extreme weather events have an impact on agriculture and crop productivity (Howden et al. 2007, Vos et al. 2022). Intensive wheat varieties with a reduced genetic diversity may displace old extensive varieties that are well adapted to environmental changes, and may be replaced by farmers using more fertilisers and pesticides in less favourable areas. Instead of overusing chemicals, the right choice of species should be made. This can be achieved by using modern varieties or by reintroducing old ones (Szalay 2009).

Nowadays, there is a growing demand from consumers and farmers to produce healthy food, which has led to a focus on organic farming (Niggli 2015, Nipers et al. 2024). Especially in the case of cereals, it is important to reintroduce old varieties with a diverse genetic stock as one of the most important food sources (Bonman et al. 2015, Wingen et al. 2017, Vikram et al. 2021). Therefore, the European Union and Hungary provide agri-environmental support to farmers growing Bánkúti 1201 winter wheat (*Triticum aestivum* L. ssp. *aestivum* cv. 'Bánkúti1201') and einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*) to conserve endangered and rare field crops of high cultural and genetic importance.

The main aim of plant breeding is to create genotypes that are well adapted to environmental changes, productive and resilient. It also aims to breed less resource-intensive varieties that are well suited to low-input extensive farming (Pieruschka & Schurr 2019, Kim 2020). Commercial and scientific plant breeding programmes typically use traditional phenotyping to evaluate variety traits, e.g. yield, biomass, plant height, or resistance to stress factors are measured using manual tools (Uzal et al. 2018, Kim 2020). These methods are very often time-consuming and labour-intensive, and usually use destructive methods to assess traits, thus providing limited resources and replications in long-term research (Busemeyer et al. 2013, Walter et al. 2019, Selvaraj et al. 2020). The development of new technologies is needed to complement and improve traditional manual breeding. This will enable rapid, accurate and reproducible phenotyping of large populations, supporting selection processes and the discovery of resilient genotypes (Niazian & Niedbala 2020).

Nowadays, a wide range of handheld and aerial remote sensing data collection tools are available. In addition to aerial monitoring, ground-based remote sensing data collection can also be used to accurately, rapidly and non-destructively survey plant individuals and large populations (Berke et al. 2006, 2010ab, Costa et al. 2020). RGB devices that detect in the visible light range have become important in agricultural data collection due to their cost-effectiveness. In addition, data obtained by remote sensing and digital image processing can provide information that can be used to plan interventions to adapt to environmental conditions (Hatfield et al. 2008).

Although the number of research and comparative studies is increasing, there is still a knowledge gap about the environmental, nutritional and health benefits of bread wheat genotypes. In addition, there is a lack of experience with ground remote sensing for breeding. The production, storage and processing of large amounts of raw data is challenging for experts, but it is a viable alternative to traditional phenotyping.

One of the main aim of the dissertation is to investigate the yield and grain quality parameters of traditional and modern winter wheat and einkorn wheat varieties under extensive cultivation. The other main objective of the dissertation is to investigate the application of digital image processing as a modern technology in wheat cultivation using ground-based and aerial remote sensing. The aim is to investigate the suitability of visible-range RGB sensors for digital phenotyping and differentiation of cereals at early developmental stages, and for early detection of drought and nutrient deficiency symptoms. The long-term goal is to use the developed technology in plant breeding programmes, particularly for the conservation of genetic resources of rare and endangered field crops and the maintenance of agrobiodiversity. To this end, I measured yield and quality parameters in field experiment and analysed RGB data of sown varieties on orthophoto, from which I calculated 16 different vegetation indices. In addition, I measured and analysed the digital geometric and RGB data of the wheat varieties studied in 225 images under laboratory conditions.

The research questions were:

1) Can old and new wheat varieties be successfully grown in low-input, environmentally friendly extensive farming?
2) Is the developed technology suitable for digital phenotyping of wheat varieties?
3) Is it possible to differentiate wheat varieties at an early stage of development using a commercially available RGB DSLR camera and digital image processing?
4) Can wheat varieties at early development stage be detected for nutrient deficiency and drought stress using a commercially available RGB DSLR camera and digital image processing?

2. MATERIALS AND METHODS

2.1 Plant material

Two winter wheat varieties and three einkonr wheat varieties were studied. (1) *Mv Magdaléna*, a cultivated winter wheat variety listed in the national variety register, bred in Martonvásár, Hungary, and (2) *Bánkúti 1201*, an old Hungarian winter wheat variety. Nowadays it is used for the breeding of modern wheat varieties due to its favourable genetic potential (Juhász et al. 2003, Balla et al. 2013). (3) *Mv Alkor* is a registered Hungarian einkorn cultivar used in organic farming, and bred in Martonvásár. (4) *Schiemann* einkorn is from Morocco and is registered (gene bank code: RCAT 074129) in the Hungarian gene bank, Plant Diversity Centre (NöDiK, Tápiószele; now the National Centre for Biodiversity and Gene Conservation). (5) The *Bözödi* einkorn landrace originates from the sub-mountainous regions of Transylvania (Romania), cultivated under traditional farming practices and it is an excellent genetic source (Szabó 1976, Péntek & Szabó, 1981, Hajnalová & Dreslerová, 2010, Gyulai 2019). The re-introduction of its cultivation is promoted by organic farming. Seeds were provided by the Plant Diversity Centre (NöDiK, Tápiószele). As a result of selection work, it was granted state variety recognition in 2022 by the Minister of Agriculture as *Szarvasgedei alakor*.

2.2 Uncontrolled field experiment

During the seed preparation, the thousand seed weight (g) of each variety was measured with a laboratory counter and then the seeding standard was determined. The experimental area was established in a soil homogeneous field of 4 plots in 400 m^2 (47°40'59.5 "N 19°40'08.2 "E). Soil preparation was done before sowing. The 1 m × 9 m experimental plots were randomly designated in the field in four replicates in Nagygombos (Hungary), rented by the Institute of Plant Production of Szent István University (now Hungarian University of Agriculture and Life Sciences).

On 17 November 2016, the three einkorn wheats (Mv Alkor, Schiemann és Bözödi) and the winter wheat Bánkúti 1201 were sown with a mechanical small plot seeder. The experimental area was treated uniformly (no use of fertilisers, herbicides or irrigation). The harvest was conducted on 21 July 2016 in the ripening stage of wheat cultivars.

After harvest, (1) the *yield* of each wheat was measured in the field and calculated per hectare. The grains with glume were hulled with a Santec SRO VKI11 laboratory huller in a laboratory to prepare samples for measuring grain quality parameters. (2) *Protein* and (3) *gluten content* were measured in four repetitions with a Mininfra-ScanT Plus near infrared optical analyser at wavelengths ranging between 790 nm and 1064 nm. (4) The *storage volume* of grains was measured in a laboratory glass in four replicates.

We conducted the aerial survey together with the staff and students of Dennis Gabor College (DGC). The experimental site (47°40'59.5 "N 19°40'08.2 "E) was flown with a DJI Phantom 3 Advanced drone on 04 March 2016 using a Canon EOS 30D RGB SLR camera with visible-light range detection. The flight start time was 9:55 am and the flight end time was 10:05 am. The flight altitude was 12 m. After the recording, the drone images were merged on DGC and the high-resolution TIFF orthophoto was delivered.

The 16-bit TIFF RGB images were analysed using ImageJ image processing software. In four replicates per species, the study areas (ROI 1-16) were manually selected and the RGB band data were measured, from which 16 RGB vegetation indices per species and per area were calculated for subsequent data analysis.

2.3 Controlled laboratory experiment

2.3.1 Experimental design

The seeds were sowen in Jiffy peat pots where they germinated for 14 days. After the plants were grown in pots for 43 days in an unheated greenhouse from 16 September 2017. After 43 days, the seedlings were planted in round pots in a 3:2:1 mixture of 2800 cm^3 of garden soil, compost and sand. The pots were placed in a Conviron PGR-15 phytotron spring/summer growth chamber offering a growth area of 1.5 m^2 and a growth height of 1450 mm. The night/day temperature was maintained at 10/15°C for 11 days. For an additional 19 days, the night/day temperature was kept at 13/17°C, illuminated with halogen lamps for 12 hours per day. The average air humidity was 75% during the night and 65% during the day (Tischner et al. 1997, Balla et al. 2013).

Three different treatments were implemented in the study: control, nutrient deficiency, and drought stress during the tillering development stage. Control pots were watered daily with tap water and supplemented once a week with Wuxal Super nutrient solution. The nutrient deficient pots were watered daily with tap water without extra nutrients. Drought stress pots were watered twice a week for 13 days and once a week for 17 days with tap water supplemented with the same nutrient solution as the control. All treatments were performed in five replicates, each cultivar was represented by 15 pots and the treatments were applied for 30 days.

2.3.2 Digital RGB image recording

I photographed the above-ground part of winter wheat and einkorn wheat at three time points to determine the geometric parameters and RGB data of the plants for digital phenotyping. I recorded 45 images per species, analysing 225 images in total. To measure the growth parameters of winter wheats and einkorn wheats, the aboveground part of the plants was photographed three times (9, 18, and 26 November 2017) after being transplanted from Jiffy into the pots in the early stage of plant growth. The plants were placed in front of a white background and illuminated with halogen bulbs. Digital images were taken of the central zone of the canopy with a Canon EOS 30D DSLR digital still color camera (2009 Canon Inc., Tokyo, Japan) with 8.2 megapixel resolution. Data were recorded in unprocessed raw format; intensity data were digitalized to 12 bits.

The camera was mounted on a tripod, and the distance from the camera tripod to the subject was constantly 3.2 m. The digital camera settings were as follows: exposure time $1 \times 10-2$, aperture f/10, ISO 100, focal length 50 mm, white balance with 4900 K, flash turned off. All images in the experiment were stored in CR2 (Canon RAW version 2 image file) format. The CR2 format contains minimally processed data from image sensors in a digital camera; the file contains white balance, saturation, contrast and sharpness settings, but delays processing. No destructive changes are made to the raw image file, and suitable for further measurements (Wang et al. 2014).

2.3.3 Digital RGB image processing

The image processing was carried out together with the Dennis Gabor College in Budapest. Adapting previous research on RGB images (Liu & Pattey 2010, Walter et al. 2019), we applied thresholding and segmentation methods to separate target objects (plant pixels) and non-target objects (e.g. background, pots, ground) in the images.

The images were processed with Adobe Photoshop CC 20.04.4 software (Adobe Systems Inc., San Jose, CA, USA). The processing was performed by the image segmentation feature of the Camera RAW 11.2 plug-in. The aim of the pre-processing was to remove any inappropriate information from the images to be measured. This semi-manual processing was preceded by further pre-processing, which included adjustments to white balance, lighting, saturation and vibrancy, shadows, dark and light tones. After parameterisation, a selection mask was created to overlay the original raw image. The canopy was separated from the white background and from the other surfaces (e.g. pot, marker, table), which is important for the accurate estimation of growth and biomass production. The processed images were saved in 16-bit TIFF uncompressed format.

The images were analysed under controlled conditions based on the projected area of the plant in the image. Image parameters were determined with the open source software ImageJ. In the RGB stack, the color threshold was adjusted according to the intermodes thresholding method. Red was selected as the threshold color in HSB color space. The method is based on the appropriate contrast between the plant and the background. The object was separated from the background pixels based on contrast.

Pixels of target objects are suitable for quantification and analysis, and provide information on the morphological traits, growth vigour and response to environmental stress of cereals. The percentage of object pixels relative to the total number of pixels in the entire area of the visual image—called the projected area—was then measured. The following parameters were calculated in thresholded images: (a) *area*: area of composite selection, ignoring pixels outside the object; values were expressed in square pixels; (b) *perimeter*: the length of the outer boundary of the composite selection; bounding rectangle: the smallest (c) *width* and (d) *height* enclosing the selection; (e) *Feret's diameter*: the maximum distance between any two points along the selection boundary, also known as the maximum caliper. The latter four parameters were expressed in pixels (Ferreira & Rasband 2012). In addition to the geometric parameters, I measured the RGB data of the images, from which I calculated 16 vegetation indices for each species and each treatment.

2.4 Data analysis

The differences between varieties, the effects of nutrient deficiency and drought stress were analysed using a general linear model, the one-way ANOVA test. In all cases the data met the prerequisites for the applicability of the test (homogeneity of variance, normal distribution of residuals). In all analyses, the test reliability was determined at the 95% confidence interval. In the analysis, several geometric parameters of the differently treated plants were examined, e.g. area, perimeter, width, height, Feret diameter. The geometric parameters of the differently treated plants were analysed one by one in separate models, where the dependent variable was the geometric trait and the fixed factors were the type of the treatment (control, nutrient deficiency, drought) or the wheat cultivars (Mv Magdaléna, Bánkúti 1201, Mv Alkor, Schiemann and Bözödi).

For more detailed results, I used Tukey's post-hoc significance test to compare the effect of treatment types on geometric parameters in pairs.

I calculated 16 different vegetation indices from the measured RGB data. The differences between the three treatments and the wheat varieties were analysed using one-way ANOVA test and Tukey's post-hoc test. Each vegetation index was tested in a separate model, where the dependent variables were the vegetation indeces and the fixed factors were the treatment types or the different wheat varieties. The analysis for the varieties was performed on both laboratory and field experiment data.

The 16 RGB vegetation index formula used in the models:

- BGI = B/G
- BI = ((R^2+B^2+G^2)/3)^2
- ExB = 1.4*B/(R+G+B)-G/(R+G+B)
- GCC = G/(R+G+B)
- GLI = (2*G-R-B)/(2*G+R+B)
- GR = G/R
- HUE = atan(2*(B-G-R)/30.5*(G-R))
- MGRVI = (G^2-R^2)/(G^2+R^2)
- MVARI = (G-B)/(G+R-B)
- RCC = R/(R+G+B)
- RGBVI = (G^2-(B*R)/G^2+(B+R))
- PRI = R/G
- TGI = G-0.39*R-0.61*B
- VEG = G/(R^0.667*B^0.334)
- vNDVI = 0.5268*((R/(R+G+B))^0.1294*(G/(R+G+B))^0.3389*(B/(R+G+B))^-0.3118)
- WI = (G/(R+G+B)-B/(R+G+B))/(R/(R+G+B)-G/(R+G+B))

From the laboratory experiment data, I also analysed the variation of the vegetation index vNDVI and the area parameter values as a function of different wheat varieties, treatment types and time. For the analysis, I used a three-way ANOVA test, where the dependent variable was vNDVI or area values, while the independent variables were wheat varieties, treatment types and time. In the model, I also examined the effects of the independent variables separately and jointly in interaction, i.e., interactions between varieties*treatment, varieties*date, treatment*date, varieties*treatment*date. In this case I applied Tukey's post-hoc analysis to investigate the effect of independent variables in more detail.

Pearson's correlation coefficient was used to determine significant relationships between grain quality and quantitative parameters (yield, protein content, gluten content, storage volume). In the laboratory experiment, I examined the correlation between area and vNDVI values of different wheat varieties, also using Pearson's correlation test. The correlation of the two studied variables was examined at the three different measurement times.

The calculation of the vegetation indices, the statistical analysis and the visualization of the data were performed in the statistical environment of RStudio, using the integrated packages of the program (stats, graphics; R Core Team 2021), the graphics package ggplot2 (Wickham 2016) and the package dplyr (Wickem et al. 2023). In addition, I used IBM SPSS Statistics 17.0 software for analysis.

3. RESULTS AND DISCUSSION

3.1 Results of the field experiment

For the yield, most varieties differed significantly at the $p < 0.001$ level. Among the studied varieties, winter wheat 1201 Bánkúti had the highest yield average (4.5 t/ha), while Bözödi einkorn had a slightly lower yield (4.4 t/ha). The lowest yields were obtained with Mv Alkor (3.8 t/ha) and Schiemann (3.5 t/ha) einkorns. The highest protein content was found in Schiemann (12.6 %), followed by Bánkúti 1201 (12.5 %), Mv Alkor (11.7 %) and finally Bözödi (9.3 %), which had the lowest protein content in the studied samples. There were no significant differences in the protein content of Bánkúti 1201 (23,6 %), Mv Alkor (22,5 %) and Schiemann (25,8 %), Bözödi einkorn having the lowest protein content (15,4 %). Schiemann einkorn had the highest storage volume (80,1 kg/hl), which was similar to Bözödi einkorn (79,1 kg/ha) and MvAlkor (77,3 kg/hl). Bánkúti 1201 (75,7 kg/hl) had the lowest storage volume.

Among the 16 vegetation indices analysed, derived from aerial RGB data, the vNDVI index was suitable for detecting the early developmental stage of the sown wheat varieties from aerial photographs. In all cases, the values were between 0.35 and 0.40.

3.2 Results of the laboratory experiment

3.2.1 Relationship between treatments and digital geometric parameters and RGB vegetation indices

For all the geometric parameters tested (area, perimeter, width, height, Feret diameter), the effect of drought was significant compared to the control pots ($p < 0.05$). The strongest impact was found for the area variable. While nutrient deficiency was not significantly found for any of the parameters compared to the control in the experimental setting ($p < 0.05$).

Of the studied 16 vegetation indices derived from RGB data, 12 indices at the $p < 0.001$ significance level were suitable for detecting differences between treatments, these were BGI, BI, ExB, GCC, GLI, GR, HUE, MGRVI, MVARI, PRI, VEG and vNDVI. While TGI at the $p < 0.01$ significance level, RGBVI at the $p < 0.1$ significance level detected differences between treatments. Two indices, RCC and WI, were not able to differentiate between treatments.

All of the above 12 indices were suitable for detecting the effects of drought. Unlike the previous results, TGI only detected the effect of drought at the $p < 0.05$ significance level and RGBVI was no longer suitable for detecting the effect of drought in wheat varieties, similarly to RCC and WI. Nutrient deficiency was not detectable by any of the studied vegetative indices compared to the control in the experimental setting ($p < 0.05$).

3.2.2 Differentiation of varieties using digital geometric parameters and RGB vegetation indices

All the geometric variables except perimeter were suitable to significantly separate winter wheat varieties from einkorn wheat varieties. The geometric parameter area was able to significantly separate Schiemann and MvMagdaléna, Schiemann and Bánkúti 1201, Mv Magdaléna and MvAlkor, MvMagdaléna and Bözödi, MvAlkor and Bánkúti 1201, and Bözödi and Bánkúti1201 (Fig. 1). In the case of width, there was a significant difference between MvMagdaléna and MvAlkor and between MvMagdaléna and Bözödi. In the case of height and Feret diameter, most varieties were significantly different: Schiemann from MvMagdaléna, MvAlkor and Bánkúti1201, MvMagdaléna from MvAlkor and Bözödi, and MvAlkor from Bánkúti1201 and Bözödi from Bánkúti1201.

Most of the vegetation indices based on RGB data were suitable to distinguish wheat varieties included in the study. 11 indices showed differences between varieties at $p < 0.001$ significance level, which were BGI, BI, ExB, GCC, GLI, TGI, MVARI, RCC, RGBVI, VEG and vNDVI. While GR, MGRVI and PRI indices showed differences between varieties at $p < 0.05$ significance level. HUE and WI indices were not suitable for detecting wheats.

The number of pairwise discrepancies detected for the indices was as follows: BGI: 6, ExB: 6, GCC: 6, GLI: 6, TGI: 6, VEG: 6; MVARI: 6, BI: 5, RGBVI: 5, vNDVI: 4, RCC: 2, GR: 1, MGRVI: 1, PRI: 1, HUE: 0, WI: 0.

The BGI, ExB, GCC, GLI, TGI, VEG and MVARI indices similarly showed differences between the winter wheat Bánkúti 1201 and the einkorns Bözödi, MvAlkor and Schiemann, and between the winter wheat MvMagdaléna and the einkorns Bözödi, MvAlkor and Schiemann. BI and RGBVI were suitable for the differentiation between the winter wheat Bánkúti 1201 and the three einkorn varieties and between MvMagdaléna and Bözödi. Furthermore, the two winter wheats were separated from each other by the BI index, and MvMagdaléna and MvAlkor by the RGBVI index. At the vNDVI index, Schiemann was separated from MvMagdaléna and Bánkúti 1201, MvMagdaléna from Bözödi, and Bözödi from Bánkúti 1201. Using the RCC index, Bánkúti 1201 could be separated from Bözödi and Schiemann. Three indices, GR, MGRVI and PRI, were only able to distinguish one species, all three indices showing significant differences between MvAlkor and MvMagdaléna.

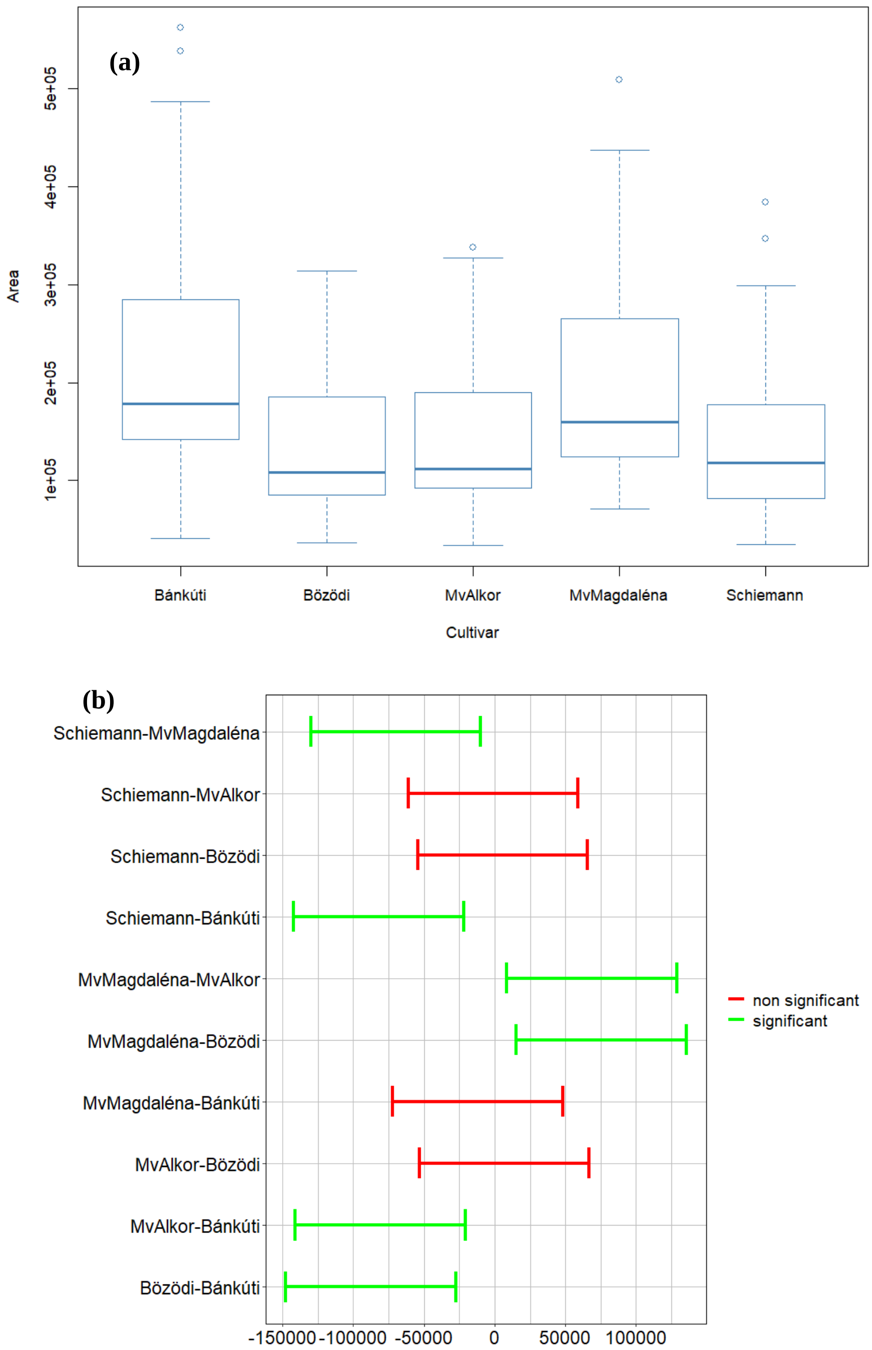

Figure 1: Detection of differences between varieties using the area parameter: **a)** area data of the studied wheat varieties, **b)** differences between wheat varieties according to the Tukey test.

3.2.3 The relationship between the most accurate predictor variable (area) and the varieties as a function of treatments and time

The general linear model showed that all the fixed factors (wheat varieties, treatments, date of recording) significantly affected all digital image parameters at the $p < 0.05$ level. Based on the number of significant interactions—F value and partial $\eta 2$—the strongest relationship with fixed factors was found for area. The fixed factors explained 91.8% of changes in area. The strongest relationship was with time, but species and treatments also significantly influenced the area variable. For all varieties, there was a steady increase in area over time ($F = 443,804$, $\eta2= 0.831$, $p < 0.05$). A significant relationship was also found between area and treatments ($F = 214,260$, $\eta2= 0.704$, $p < 0.05$). There was a moderately strong relationship between area and species ($F = 67.243$, $\eta2= 0.599$, $p < 0.05$) and between area, time and treatments combined ($F = 75.366$, $\eta2= 0.626$, $p < 0.05$).

According to Tukey's post hoc significance test, significant ($p < 0.05$) differences were found between all dates of recording and all treatments. Area increased with time for all varieties. Even at small time scales, changes were detectable in the early developmental stages of wheat. As for the varieties, Mv Magdaléna and Bánkúti 1201 had a significantly higher area than Mv Alkor, Schiemann and Bözödi. Mv Magdaléna and Bánkúti 1201 were significantly different from the other varieties in the first two dates, but the growth of Mv Magdaléna slowed down by the third measurement and became similar to Mv Alkor, Schiemann and Bözödi.

Drought had a negative impact on growth, resulting in a significantly lower area than control or nutrient deficiency with time. The varieties reacted to stress in a slightly different way. Mv Magdaléna and Bánkúti 1201 had a significantly bigger area affected by drought than the other varieties. Nutrient deficiency had no impact on growth with time compared with control, nor did the varieties react to nutrient deficiency significantly.

3.2.4 Examination of the vNDVI index

By statistical analysis of the vNDVI index (Costa et al. 2020), derived from one of the most important vegetation index (NDVI) used in agriculture, it can be shown that the vNDVI value differs significantly between species and treatments at all measurement times in the laboratory study. Among the interactions, the joint effect of treatment and time of measurement and the joint effect of species, treatment and time of measurement were significant according to the ANOVA test.

There was a positive relationship between the area parameter and the vNDVI values at the different measurement times: weakly positive at the first measurement, strongly positive at the second and third measurement. The effect of drought could be detected by examining the RGB and geometric data together, as there was a negative significant relationship between vNDVI values and the area parameter for MvMagdaléna, MvAlkor and Schiemann under drought treatment.

3.3 Discussion

3.3.1 Cultivation of old and new wheat varieties in low-input, environmentally friendly extensive farming

Although the number of comparative studies about old and new types of wheats is increasing, there are still relatively few research groups working in this area (Shewry 2018), so only a limited number of studies on different genotypes are available. Therefore, one of the aims of this doctoral study was to investigate yield and grain quality parameters of traditionally and modernly bred winter wheat and einkonrn varieties.

According to the study of Morris & Sands (2006) modern bread wheat differs in yield and in nutrition benefits from old types of wheats, but these differences have not been confirmed by detailed analysis (Ribeiro et al. 2016, Dinu et al. 2018, Shewry 2018). Ruiz et al. (2019) demonstrated that old wheat varieties show yields too low to be competitive in terms of crop productivity. Hidalgo et al. (2009) reported 0.75–2.5 t/ha yield of einkorns and 3.5–6.7 t/ha yield of bread wheat. The grain yields obtained in the present work are in line with the Hungarian study of Bencze et al. (2020) where the yields of einkorn landraces were around 3 t/ha. In other European countries (e.g., in Italy) 0.84–4.5 t/ha yields were recorded for einkorn (Castagna et al. 1995). Similarly, our study shows that modern wheats have better yield production compared to old and ancient wheats, but the latter are not far behind. Despite the lower yield, they provide acceptable yield under ecologically sustainable low-input management. Old winter wheat Bánkúti 1201 (4.5 t/ha) and the three einkorns had acceptable yields (Bözödi 4,4 t/ha, Mv Alkor 3,8 t/ha, Schiemann 3,5 t/ha) for low-input and organic farming.

Wheat nutritional value and health benefits rather than yield could be an important driver for the reintroduction of ancient wheats (Morris & Sands 2006). According to the study of Zaharieva et al. (2014) and Van Boxstael et al. (2020) the nutritional value of ancient wheats is excellent. Bencze et al. (2020) revealed that the grain protein content of einkorn Mv Alkor was less than 15%. In contrast Hidalgo et al. (2009) reported higher total proteins (17.7–20.5%, on average +59%) in einkorn wheat than in bread wheat under standard cultivar practices and in organic farming, where the genotype and the yearly environmental variation exerted major effects on protein content. In the review of Hidalgo & Brandolini (2014) *T. monococcum* kernels had a high protein content, on average 18.2%, but slightly lower concentrations (10–17.4%) are reported by some other authors. In our study the average yield and grain quality were satisfactorily high both in bread wheat and in einkorn wheat, although the relative impacts of the genotypes, environmental factors and differences in cultivation should also be investigated further.

Gluten intolerance is a widespread problem nowadays, and this is the other reason why ancient wheats are being rediscovered as a healthy food (Charmet 2011, Zaharieva & Monneveux 2014). We found that all of the studied einkorns had a significantly lower gluten content than winter wheat cultivars: Bözödi 15.4%; Mv Alkor 22.5%; Schiemann 25.8% compared to Bánkúti 1201 23.6% and Mv Magdaléna 31.8%. Therefore, we agree that einkorn gluten content has poor bread manufacturing properties; nevertheless, einkorn flour is ideal for preparation of healthy cookies and produces good-quality pasta (Brandolini & Hidalgo 2011, Hidalgo & Brandolini 2014).

3.3.2 Use of digital image processing for phenotyping wheat varieties

Another aim of the doctoral research was to phenotype and differentiate wheat varieties using a more cost-effective and simpler measurement method than multi- and hyperspectral remote sensing, and to detect and monitor symptoms of drought and nutrient deficiency in the early developmental stages of the plants. For this purpose, I adapted for the first time an image processing technique for einkorn wheat, using real-time RGB image acquisition and digital image analysis to study control, nutrient-deficient and drought-treated plants. Using statistical methods, I showed that area was the most important geometric variable for detecting differences and drought responses between varieties among the studied parameters, and that most of the vegetation indices derived from RGB data also provided good estimates of the responses of varieties to drought stress and the differences between winter wheats and einkorns.

Determining geometric and spectral parameters in a non-invasive, non-destructive way, and thus estimating plant condition and yield, is an important task in both modern plant breeding and phenotyping (Busemeyer et al. 2013, Pieruschka & Schurr 2019, Kim 2020, Omari et al. 2020, Brainard et al. 2021) and in the precision agriculture (Lu et al. 2019). In our case, above-ground biomass/green weight is most accurately expressed by the area parameter, which is a widely used as agronomic indicator to characterize crop growth and nitrogen content and to estimate crop yield (Acorsi et al. 2019, Lu et al. 2019). Previous studies have shown that estimated canopy cover based on RGB images was strongly correlated with above-ground biomass (Lee & Lee 2013, Bendig et al. 2015, Cen et al. 2019), and this was also true under a wide range of environmental conditions (Liu & Pattey 2010).

In the laboratory experiment, the strongest relationship was as a function of time, showing the growth vigour of the varieties, and even over a short time period, this change was detectable in the early developmental stage of the wheat varieties. Thus, our results are in line with the above studies, that by digital image processing and analysis of appropriate geometric parameters and RGB data, grain growth can be measured, showing a strong correlation with biomass production and stress response to drought stress.

3.3.3 Differentiation of wheat varieties at an early stage of development using RGB camera and digital image processing

Analysis of digital data of plant parameters (e.g. growth rate, leaf area, etc.) can provide important indicators of genotype variability (Fanourakis et al. 2014, Wang et al. 2014, Golbach et al. 2016, Zhang et al. 2018) and help to understand the differences between varieties and genotypes (Kim 2020). Several techniques have been developed to estimate yield based on plant biomass in different crops (Bendig et al. 2014, Li et al. 2015, Iqbal et al. 2017, Acorsi et al. 2019). Golbach et al. (2016), Bendig et al. (2014) and Iqbal et al. (2017) revealed that plant height or Feret's diameter can be used for estimating crop structure parameters and for predicting above ground biomass. Based on the literature survey, no study has addressed the joint analysis of the relationships between geometric and RGB data and between winter wheat and einkorns.

In the laboratory study, all the geometric variables (area, width, height and Feret diameter) except perimeter were suitable to distinguish winter wheat varieties from einkorn wheat varieties. Moreover, 11 vegetation indices showed differences between varieties with precision $p < 0.001$, which were BGI, BI, ExB, GCC, GLI, TGI, MVARI, RCC, RGBVI, VEG and vNDVI. In the controlled experiment, there was a positive relationship between the area parameter and vNDVI values at different measurement times, while in the field experiment, the vNDVI index was suitable to detect the early developmental stage of seeded wheas. The results show that vegetation indices derived from geometric and RGB data are suitable for detecting differences between wheat varieties and indirectly above-ground biomass.

Furthermore, the results show that the use of combined information to estimate the performance of wheat provides a better and more accurate estimation than a single indicator used. It is hoped that the developed measurement method and data analysis can be successfully applied in various plant breeding programs, especially for ancient wheat varieties and other field crops for rapid extraction of phenotypic information and for qualitative and quantitative prediction of varieties.

3.3.4 Detection of drought stress in early development of wheat varieties using RGB camera and digital image processing

The only treatment with a significant negative impact on growth was drought stress. Under controlled conditions, the effect of drought was detectable for all the geometric parameters tested (area, perimeter, width, height, Feret diameter), with significantly lower values for drought-treated individuals. The area parameter was the best growth indicator to detect the response of varieties to drought. Drought treatment resulted in much slower growth over time compared to control and nutrient deficient individuals. Among the studied cultivars, the two winter wheats performed best in both growth and resistance to drought stress. But over time, winter wheat was more affected by drought than the einkorns. Although several studies (Guzmán et al. 2009, Hajnalová & Dreslerová 2010, Zaharieva & Monneveux 2014) found that einkorn wheats were highly resistant to environmental stresses, we, on the contrary, found that the studied einkorns responded worse to drought stress in the tillering stage than winter wheats. Later the response to environmental stress caused by water deficit was reduced. Mv Magdaléna and Bánkúti 1201 had significantly higher growth rate under drought stress than the three einkorn wheats: Mv Alkor, Schiemann and Bözödi. The probable reason for this is that the studied winter wheats have satisfactory climatic resistance and extremely good drought tolerance (Juhász et al. 2003, MTA ATK 2015, Sehgal et al. 2018).

In addition to the geometric parameters, the effects of drought on the early developmental stage of cereals under laboratory conditions could be detected and monitored using RGB data. Among the 16 vegetation indices derived from RGB data, 12 indices (BGI, BI, ExB, GCC, GLI, GR, HUE, MGRVI, MVARI, PRI, VEG and vNDVI) were able to detect drought symptoms even at short time scales (one month). The effect of drought could also be detected by a combined analysis of RGB and geometric data, as drought treatment significantly decreased the vNDVI value in three cultivars (MvMagdaléna, MvAlkor and Schiemann).

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 The role of old cultivars in maintaining agrobiodiversity

Based on field study and literature I conclude that both winter wheat and einkorn wheat can be grown successfully under low-input and environmentally sustainable organic farming and still keep their yield and quality. Ancient wheats and old landraces represent not only a key reservoir of genetic diversity in crop breeding, but they also play an important role in maintaining the biodiversity of agroecosystems. The cultivation of various species could provide a more diverse and sustainable agriculture, instrumental in adapting to climate change, as they do not require irrigation, yet provide a safe yield production. Further research is then strongly recommended, particularly on a wider range of genotypes of old and modern wheat cultivars, helping to conserve and protect agroecosystems in the long run.

4.2 The imaging technology in digital phenotyping

Aerial and aboveground imaging technologies may help private breeding programs and research institutes in moving from conventional phenotyping to novel approaches. The main bottleneck is achieving a reliable imaging solution that deals with image variability due to environmental uncertainty and limited analytics (Walter et al. 2019, Kim 2020). Here, I adapted a RGB-based analysis method that offers a time-saving, non-destructive alternative in a controlled environment.

Under controlled conditions, digital image analysis and vegetation indices derived from geometrical and RGB data were used to detect and monitor drought effects and differences in the early developmental stage of cereals. Differences were detectable even at small time scales (one month). For the field experiment, the vNDVI index was suitable to detect the early developmental stage of the wheat varieties based on information collected by remote sensing.

The results suggest that the combination of RGB-based imaging technologies and traditional plant breeding and manual phenotyping can cost-effectively contribute to the breeding of resilient genotypes. Furthermore, the analysis of digital geometric parameters and RGB data may be a viable choice for the rapid prediction of above-ground biomass production at cereals and the detection of drought stress at early developmental stages. It can also improve the prediction of crop performance, which is key to maintain crop security in the ongoing changing climate.

Compared to conventional plant breeding methods, we have developed a non-destructive and real-time method for detecting and monitoring drought stress, and differences between winter wheat and einkorn wheat, and estimating their growth under controlled conditions at early developmental stages. The technology developed is a cost-effective system: open source software (ImageJ) and a commercial DSLR camera (Canon RGB camera). It is a fast, non-invasive and economical, easy-to-use technique and can be applied easily, whereas conventional laboratory instruments and multi- and hyperspectral sensors are expensive and time-consuming to operate. Our results are expected to be applicable in various plant breeding programmes for rapid extraction of phenotypic information of wheat genotypes.

4.3 Suggestions for improvement

Part of the research was carried out during the initial phenological stages of growth (germination, emergence and tillering) so further investigation of the reproductive stage of the cereals is highly recommended. At present, much of the developed methodology is limited to studying potted plants in a controlled environment chamber. Further validation is necessary to ensure their application in the field. Hence standard controlled processes need to be developed to make them acceptable in proper agricultural application. The use of automated Artificial Intelligence (AI) based systems to facilitate and accelerate data processing is a further development direction. The development of an AI-based approach would provide the opportunity to process imagery and numerical data from large sample sizes more efficiently.

5. NEW SCIENTIFIC RESULTS

1. I have demonstrated by field experiment and literature data that the studied wheat genotypes can be grown successfully under extensive conditions if the aim is to cultivate in an environmentally friendly organic agriculture.

2. Based on RGB images, using a combination of image processing techniques applied in practice, I determined the parameters most sensitive to the digital phenotype of winter wheat and einkorn wheat varieties.

3. I have adapted a new method to record the geometric and RGB characteristics of winter wheat and einkorn wheat varieties at an early stage of plant development.

4. I have demonstrated that the developed technique is suitable for documenting the growth dynamics of cereals even at short time scales.

5. I have experimentally demonstrated that the developed technique is suitable for detecting signs of water deficit in cereals at an early stage of development.

6. I built up a digital parameter library based on the specific developmental dynamics of the varieties recorded under experimental conditions. Based on the digital parameter library, varieties can be differentiated at an early stage of development.

7. I have shown that vegetation indices derived from RGB data can be used to separate winter wheat and einkorn wheat varieties at an early stage of development.

6. PUBLICATIONS RELATED TO THE THESIS

IF journal article:

Csákvári, E., Sáradi, N., Berki, B., Csecserits, A., Csonka, A.Cs., Reis, B.P., Török, K., Valkó, O., Vörös, M., Halassy, M. (2023): Native species can reduce the establishment of invasive alien species if sown in high density and using competitive species. *Restoretion Ecology*, 31(5):e13901, D1, IF=2,8.

Tanács, E., Vári, Á., Bede-Fazekas, Á., Báldi, A., **Csákvári, E.**, Endrédi, A., Fabók, V., Kisné Fodor, L., Kiss, M., Koncz, P., Kovács-Hostyánszki, A., Mészáros, J., Pásztor, L., Rezneki, R., Standovár, T., Zsembery, Z., Török, K. (2023): Finding the Green Grass in the Haystack? Integrated National Assessment of Ecosystem Services and Condition in Hungary, in Support of Conservation and Planning. *Sustainability*, 15(11), 8489, Q1, IF=3,251.

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Csákvári, E., Halassy, M., Enyedi, A., Gyulai, F., Berke, J. (2021): Is einkorn wheat (*Triticum monococcum* L.) a better choice than winter wheat (*Triticum aestivum* L.)? Wheat quality estimation for sustainable agriculture using vision-based digital image analysis. *Sustainability*, 13(21):12005, Q1, IF=3,251.

Bánfalvi, Zs., **Csákvári, E.**, Villányi, V., Kondrák, M. (2020): Generation of transgene-free PDS mutants in potato by Agrobacterium-mediated transformation. *BMC Biotechnology* 20:25, Q1, IF=2,387.

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Non IF (peer-reviewed) journal article - Foreign language:

Csákvári, E., Gyulai, F., Baktay, B., Berke, J. (2017): The role of environmental research in education based on digital image and metadata. *Journal of Applied Multimedia*, 1,12.

Non IF (peer-reviewed) journal article - Hungarian language:

Szitár, K., Csőszi, M., Vaszócsik, V., Schneller, K., Csecserits, A., Kollányi, L., Teleki, M., Kiss, D., Bánhidai, A., Jáger, K., Petrik, O., Pataki, R., Lehoczki, R., Halassy, M., Tanács, E., Kertész, M., **Csákvári, E.**, Somodi, I., Lengyel, A., Gallé, R., Weiperth, A., Konkoly-Gyuró, É., Máté, K., Keszthelyi, Á.B., Török, K. (2021): Az országos zöldinfrastruktúrahálózat kijelölésének módszertana többszempontú állapotértékelés alapján. *Természetvédelmi Közlemények*, 27:145–157.

Full-text publication in conference proceedings:

Csákvári, E., Enyedi, A., Gyulai, F., Berke, J. (2020): A digitális képfeldolgozás alkalmazási lehetőségei mezőgazdasági kutatásokban. In: Berke, J. (eds.) *26th Multimedia in Education Online Conference Proceedings*, pp. 104-107.

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