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Original Research Paper

Phenotyping of winter triticale canopy density in field conditions using an RGB camera

Fenotypowanie zagęszczenia łanu pszenżyta ozimego w warunkach polowych przy użyciu kamery RGB

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Triticale (× *Triticosecale* Wittmack) is a hexaploid species obtained by crossbreeding of wheat and rye. It is characterized by high adaptability to unfavorable environmental conditions, an essential feature in a changing climate. In this work, we present the results of automatic phenotyping of canopy density, a yield-forming factor, in autumn and spring (BBCH phases 22-29) for twelve commercial cultivars of winter triticale from the PDO trials (post-registration variety testing), COBORU (Research Centre for Cultivar Testing) experiments. Two field replicates, grown at two agrotechnical levels (A1, A2), were phenotyped using the HTPP (High Throughput Plant Phenotyping) platform, PlantScreen (PSI, Drasov, Czech Republic), equipped with a high-resolution RGB camera. The obtained photos were processed using MorphoAnalyser software, which is dedicated to processing recorded images and is included in the platform. The obtained results (green color pixels in the photo) and the yield were subjected to statistical analysis using Doriane software, a statistical package for plant breeding. Since the differences between the results obtained at A1 and A2 levels were not statistically significant, the data were averaged, and Pearson's correlations of canopy density in autumn and spring with yield were calculated. In both seasons, the correlation coefficients had high, positive values; were amounted to 0.79.

Keywords: field imaging, high throughput phenotyping, plant breeding, *Triticosecale*, yield potential

Pszenżyto (× *Triticosecale* Wittmack) jest heksaploidalnym gatunkiem powstałym w wyniku krzyżowania oddalonego pszenicy i żyta. Charakteryzuje się dużą zdolnością przystosowawczą do niekorzystnych warunków środowiskowych co jest istotną cechą w zmieniającym się klimacie. W tej pracy przedstawiamy wyniki automatycznego fenotypowania zagęszczenia łanu, czynnika plonotwórczego, jesienią oraz wiosną (fazy BBCH 22-29) dla dwunastu komercyjnych odmian pszenżyta ozimego z doświadczeń PDO (Porejestrowe Doświadczenie Odmianowe), COBORU (Centralny Ośrodek Badania Odmian Roślin Uprawnych). Fenotypowano dwa powtórzenia polowe, uprawiane na dwóch poziomach agrotechniki (A1, A2), wykorzystując platformę HTPP (High Throughput Plant Phenotyping), PlantScreen (PSI, Drasov, Czechy), wyposażoną w wysokorozdzielczą kamerę RGB. Uzyskane zdjęcia przetworzono przy użyciu oprogramowania MorphoAnalyser dedykowanego do przetwarzania zarejestrowanych obrazów i będącego wyposażeniem platformy. Wyniki oraz plon poddano analizie statystycznej przy użyciu oprogramowania Doriane, statystycznego pakietu dla hodowli roślin. Ponieważ stwierdzono, że różnice pomiędzy wynikami uzyskanymi w warunkach A1 i A2 nie są istotne statystycznie, dane uśredniono i wyliczono korelacje zagęszczenia łanu jesienią i wiosną z plonem. W obu sezonach współczynniki korelacji miały wysoką, dodatnią wartość; wyniosły 0,79.

Słowa kluczowe: hodowla roślin, obrazowanie terenowe, potencjał plonowania wysokoprzepustowe fenotypowanie, *Triticosecale*

Introduction

The growing demand for food, feed, and plant products as raw materials for industry and renewable energy, as well as economic conditions on the one hand and, on the other hand, limitations resulting from the need to implement European environmental protection regulations, make it necessary for crop breeding to constantly increase the productivity of cultivars introduced to the market while reducing the costs of their cultivation. Costs are understood not only in terms of financial outlays but also in terms of minimizing the impact of crops on agroecosystems by reducing the consumption of water (used for irrigation and naturally available), fertilizers, and plant protection products, in accordance with the European Green Deal initiative and the Farm to Fork Strategy (European Commission, 2020). Under these conditions, breeding progress requires the production of new cultivars resistant to diseases, pests, and abiotic stresses. This should contribute to minimizing the use of chemicals and, thus, their environmental impact. Water consumption and pollution are also important (Rybka and Nita, 2014).

Understanding the complex processes of plant development is based on the interactions between genetic information and the environment and how

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external conditions influence the plant phenotype, resulting in agriculturally desirable traits (Pieruschka and Lawson, 2015). To solve this issue, knowledge about the relationship between genotype and phenotype is needed, and this is currently a great challenge for all fields of natural sciences (Großkinsky et al., 2015). On the part of practitioners and breeders, there are expectations for the development of bioinformatics and systems biology, which will enable the construction of a virtual model of the plant, allowing for the analysis of biochemical processes and changes in gene expression in silico at each stage of growth, translating into the practical use of this knowledge (Daloso and Williams, 2021, Long et al., 2008; Ndour et al., 2017). Currently, the Japanese KEGG model (Kyoto Encyclopedia of Genes and Genomes) is one such model that allows the understanding of non-obvious relationships at the cellular and whole organism levels. However, transferring this knowledge into practice is nearly impossible (Kanehisa Laboratories, 2024). The acquisition of genetic information through sequencing and microarrays has reached a high level (Sun et al., 2020). Interpreting these data based on currently created pangenomes is a promising prospect (Hurgobin and Edwards, 2017, Zhang et al., 2023). The data collected in this way must be supplemented with precise phenotypic data.

Plant phenotyping involves the visual assessment of plants during a defined growing season and is still most often carried out by individual breeders during field observations. The human eye can be unreliable, especially after many hours of work in extreme weather conditions. Therefore, observations using appropriate cameras are able to make the quality of the collected data independent of the observer's error, which is their important feature. This would make it easier to compare results collected by different people in different locations, under different environmental and weather conditions. Therefore, an important branch of modern breeding is the development of automated phenotypic assessment both in the field and in the greenhouse. The data collected in this way is digitized and always available for viewing. Research using high-throughput plant phenotyping (HTPP) is developing rapidly, and the number of publications related to it amounted to approximately 2,000 in 2020 (Ninomiya, 2022). In the last decade, strong research centers have been established, including: in Australia: Australian Plant Phenomics Facility (https://www.plantphenomics.org.au), in Germany in Jülich: Plant Phenotyping Center (https://www.fzjuelich.de/ibg/ibg-2/EN/Research/Research Groups/JPPC/JPPC_node.html), and in Gatersleben: Automated Plant Phenotyping (https://www.ipk-gatersleben.de/en/ infrastructure/phenotyping), in Great Britain: National Plant Phenomics Center (https://www.plant-phenomics.ac.uk), in Canada: Plant Phenotyping and Imaging Research Center (https://p2irc.usask.ca) and Plant Phenomics Center in China (http://pprcen.njau.edu.cn). A platform for international research cooperation and networking has also been created, the International Plant Phenotyping Network (IPPN) (https://www.plant-phenotyping.org) (Rybka, 2018, Rybka, 2023). HTPP in field conditions is much more difficult to implement due to variable environmental conditions: light and shade, wind moving the plants, the field, and not individual plants. Progress in the development of technologies and computational techniques influences the development of fast, efficient, non-destructive, non-invasive, quantitative, repeatable and objective phenotyping methods also in field conditions. Therefore, it is possible to assess completely new features, such as the growth dynamics of plants of each genotype, in the case of regular phenotyping of breeding materials during the growing season (Ninomiya, 2022).

However, despite its dynamic development, HTPP is mostly used in university centers and research institutes, rarely in routine breeding processes. There are still no standard methods for processing data generated by HTPP and integrating them with classically collected field book data in one set, multi-stage data processing processes could be completed by the breeder in one step ("click") without the need for the participation of a specialist in data processing (Ninomiya, 2022).

In this article, we present the results of canopy density phenotyping based on RGB photos registered at Plant Breeding Strzelce, using the HTPP platform, PlantScreen System (PSI, Drasov, Czech Republic). Plants were identified in the recorded photos based on the RGB color index using dedicated, intuitive MorphoAnalyser software (PSI, Drasov, Czech Republic). Breeders expect that the automation of canopy density phenotyping will make the assessment more objective and that including it in the selection criteria may speed up the process of producing new, high-yielding cultivars (Mir et al., 2019). Of the three main components of grain yield, thousand-grain weight is routinely monitored in breeding practice due to the ease and speed of measurement and good correlation with yield (Matysik et al., 2007). The density of the canopy, and the number of ears in a plot, are rarely used parameters.

Materials and Methods

Plant material

Twelve cultivars of winter triticale (× *Triticosecale* Wittmack), cultivated in two field replicates, as part of the Post-Registration Variety Experiments (PDO) at two levels of agrotechnics A1 (average) and A2 (high) (Drzazga et al., 2013, Rozbicki et al. in., 2021) at Plant Breeding

Strzelce Ltd. Co. IHAR Group, Poland (GPS: 52.31 N, 19.41 E), on 48 experimental plots in total. The experiment was established on a plot of brown soil of the III a class, with pH of 6.5 and a high content of NPK macroelements (Bednarek, 2011, Uggla, 1981). The following cultivars were tested: Belcanto, Dolindo, Gringo, Meloman, Octavio, Orinoko, Porto, SU Liborius, Tadeus, Toro, Trapero, Trefl. Sowing was carried out on October 9th, 2019, sowing 350 germinating seeds per 1 m^2 on plots with an area of 10 m^2 (1 m × 10 m), enabling the passage of a tractor with an installed HTPP platform. The average level of agrotechnical treatments (A1) included one-time fertilization of 300 kg·ha^{-1} of NPK before sowing on September 7th, 2019. Chemical plant protection was limited to pre-sowing seed treatment and a single use of herbicides, October 18th, 2019. For the intervention, insecticides and rodent control agents were used, the same in the A1 and A2 systems. At level A2, additional nitrogen fertilization (40 kg·ha^{-1}) was applied in the multi-component foliar fertilizer Basfoliar 2.0 36 Extra (ADOB® Sp. z o. o., Poznań), protection against lodging (one treatment at the end of tillering on April 17, 2020) and protection against diseases (two treatments: April 18th and May 20th, 2020). During the growing season, the following features were visually assessed: the condition of the plantation before the winter, overwintering, earing date, as well as disease resistance and yield parameters: thousand-grain weight (MTZ) (g), yield (kg per plot, t·ha^{-1}). HTPP phenotyping was performed in the Autumn (BBCH 22-24) and Spring (BBCH 25-29): the state of plant branching was assessed on November 18th, 2019, on 48 plots (a total of 144 photos were taken), overwintering on April 30th, 2020, also on 48 plots.

Weather data

Weather data were collected by the Atmesys Agro weather station (ATMESYS atmosphere monitoring systems, Zgierz, Poland), located at the place of the experiment. Average temperatures and total rainfall in decades, in each month for the growing season, from August 2019 to July 2020, are presented against the background of multi-year data from 2007-2021 (Fig. 1).

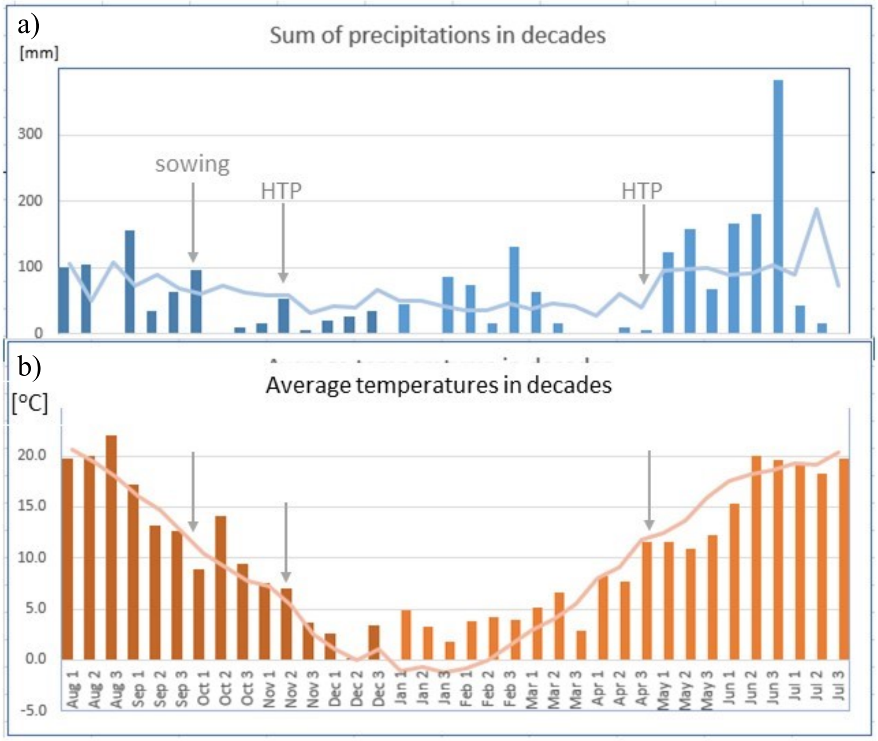

Fig. 1. (a) Total precipitation (mm) and (b) average temperatures (°C) in the 2019-2020 growing season, in decades starting from August 2019 to July 2020 (bars), against the multi-year background 2007-2021 (lines). Vertical arrows mark the sowing term and the HTP phenotyping.

Preparing the field and map for automatic phenotyping

The first stage of HTPP in field conditions was planning the field layout based on the PDO nursery's planned location, the blocks' size, and the rows' width. After the plants emerged, the so-called 0 aimed to prepare a map of the experiment by marking the location points of the plots and the places of recording photos within the plot using GPS navigation (geographic coordinates). Three photos were planned to be taken at each plot. Location points (named with a single and unique name) are crucial for high-throughput phenotyping; they are reference points for each phenotyping date during the growing season.

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Image acquisition

After carrying out the preliminary tasks, field observations were planned in the PlantScreen Scheduler program, where it was marked which points were to be phenotyped and at what date. The work plan was accepted and saved on the server in a format suitable for downloading by the computer controlling the HTPP platform. The diagram was loaded from the server to the platform using a wireless Wi-Fi network. The so-called ride 1. is to image plants and perform a phenotypic assessment using the platform at GPS points planned when making the map. For this task, the FiledScreen Client applications had to be launched, and all the rest of the imaging activities were performed automatically. The only need was to drive the vehicle around the experimental block. After completing that, the recorded photos were saved so that after arriving near the server, the images could be sent to the server via a wireless Wi-Fi connection.

Automated RGB phenotyping

Automated RGB phenotyping was performed using a FieldScreen System (PSI, Photon Systems Instruments, Drasov, Czech Republic, https://psi.cz/) mounted on a field tool carrier (Zürn 540, Zürn Harvesting GmbH & Co. KG, Germany) (Fig. 2). During phenotyping, the tractor moved in the south-north direction at a speed of 2 km·h^{-1}. In each field, three photos were taken from a distance of 0.9 m without stopping the tractor; each frame covered 0.92 m^2 of the field. RGB camera specifications (PSI, Drasov, Czech Republic): 12.36 megapixel resolution with 1.1-inch CMOS sensor (Sony IMX-253LQR-C). The sensor provided a resolution of 4112 × 3006 pixels and had a shutter function (global shutter; max fps in accessible mode, 2; pixel size, 3.45 µm). Lens specifications: computer model, 1628-MPY; focal length 16 (mm); aperture, 2.8 (Stefański et al., 2024).

Fig. 2 Field-Screen System (PSI) mounted on the Zurn 540 tool carrier, (a) view of the experimental fields where RGB phenotyping was carried out; (b) fragment of the field map with planned photo-taking locations. The arrow indicates the north direction; (c) entering the field through the sowing strip; (d) canopy density phenotyping.

Preparation of photos of canopy density using dedicated software

MorphoAnalyser software, which is part of the HTPP system, PlantScreen System (PSI, Drasov, Czech Republic, https://psi.cz/), was used to process the recorded photos. Working with MorphoAnalyser is intuitive and resembles working with publicly available, commercial graphics programs. 10 reference photos were selected, with 10 color points marked for plants and for the background. The program defined color indexes according to the RGB color system. Fig. 3 shows an example of color indexing for one photo (Rapid Tables, 2024). Because plants and background colors may vary between photos, the software suggests the most appropriate index for a specific set of photos based on the images indicated as examples. Color segmentation is also performed to reduce the number of colors using k-means clustering, which classifies colors based on similarity. The software calculates each pixel separately to create a mask. Then, it uses thresholding of the computed value and several other techniques, such as a median filter, to count pixels corresponding to plants and eliminate background pixels (Padmavathi and Thangadurai, 2016). The result of the photos processed this way is the number of pixels corresponding to the plants in the frame; the results are presented as arbitrary units (1 a.u. = 0.1 Mpix·0.92 m^{-2}).

Plant mask			Colour	Background mask			Colour
R	G	B		R	G	B	
54	97	71		32	29	70	
159	181	135		51	73	61	
192	213	146		46	37	32	
134	158	82		21	20	16	
163	184	127		36	33	28	
141	160	115		46	41	35	
56	74	62		28	27	23	
104	136	85		216	178	139	
62	77	46		36	31	27	
67	86	56		216	191	160	

Fig. 3 The share of RGB (red green blue) colors in the colors marked as a plant and as a background in one of the 10 photos used to define the plant and background masks.

Statistical analysis

The statistical analysis of the obtained results was based on analysis of variance (ANOVA). The cultivars were tested in the aspect of yield (t·ha^{-1}) and canopy density in autumn and spring, (expressed in arbitrary units: Mpix per unit area). The analysis was carried out using RnDExp software, developed by the French company DORIANE and intended for agronomic and biological research, supporting the creation of new plant cultivars and management of technical information. Illustrations of the obtained results are presented in the form of box-and-whisker plots (Doriane, 2024); show minimum, maximum and quartile values. The analysis of Pearson correlation coefficients was performed in an MS Excel spreadsheet using the XLMiner Analysis ToolPak.

Results and Discussion

The experiment was carried out in accordance with the principles of agrotechnics; there were no significant deficiencies in emergence and no damage by pests, and the plots were maintained on an ongoing basis in compliance with the requirements for agrotechnical levels. Weather conditions in autumn in terms of temperatures were at the multiannual average level, except for the second decade of October, which was 5 degrees warmer than the average (14°C vs. 9°C), which could have had a positive impact on seed germination (Fig. 1). There was variation in the tested cultivars in terms of seed vigor and germination strength, which resulted in different plot densities (Fig. 4 and Fig. 5).

The winter was abnormally warm; the average temperature in December-February was 3°C compared to 0°C recorded for many years. The spring temperature, March-April, was standard (Fig. 1). However, rainfall totals were lower - in autumn, from the second decade of October to the first decade of November, 13% of the average rainfall for the multiannual period (192 mm) fell, which was to some extent compensated by rainfall at the beginning of autumn. Sowing was carried out at the beginning of October so that the seeds had good conditions for germination. The spring was also dry, with only 15% of the average multiannual rainfall (from the second decade of March to the end of April, the average multiannual rainfall was 216 mm) and then wilting of plants of some genotypes was observed (unpublished data). In spring, it was also possible to observe genotype-dependent damage to plants after winter, poorer conditions, and health of some genotypes, which were compensated mainly by plants at later stages of vegetation (unpublished data). Noticeable differences in plot filling and changes in plant population after winter were especially visible when comparing photos of less dense plots (Fig. 4b, Fig. 5b), which was undoubtedly influenced by weather conditions (Bednarek et al., 2011, Oleksiak et al., 2022, Skłodowski and Bielska, 2009, Uggla, 1981). The significance level of differences between genotypes was $p \leq 0.05$ for yield and $p \leq 0.11$ for canopy density (Tab. 1).

The statistically assessed differentiation of the examined cultivars was poor, especially in terms of canopy density. Soil variability could also have had an impact on this score. Currently, we only have soil classification based on chemical analyses performed in accordance with the Polish Standard PN-R-04031-1997 every few years. The obtained result indicates that it would be valuable to scan fields in parallel before setting up experiments to assess soil variability as a factor influencing the final effect, yield and yield stability, and invariable determinants of the market value of cultivars (Austin, 1993). Real-time soil scanning for given GPS coordinates, an element of precision agriculture, uses the soil's electromagnetic and electrical conductivity measurement techniques. A map is prepared of the content of organic matter and water capacity of the soil, as well as other parameters, such as nutrient content, salinity,

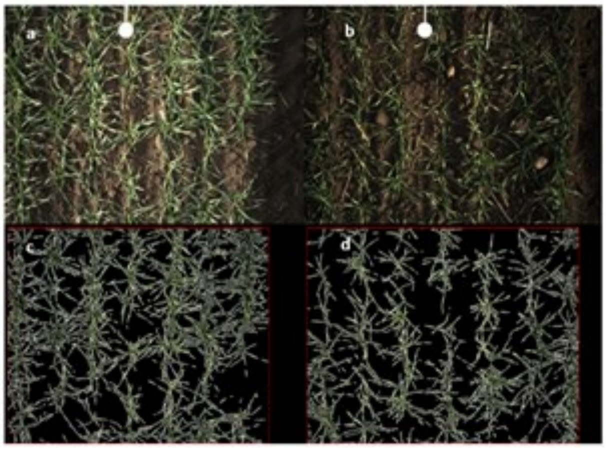

Fig. 4. HTPP of canopy density in autumn: original photos (a) well and (b) less dense canopy and the same photos processed using MorphoAnalyser software (c, d).

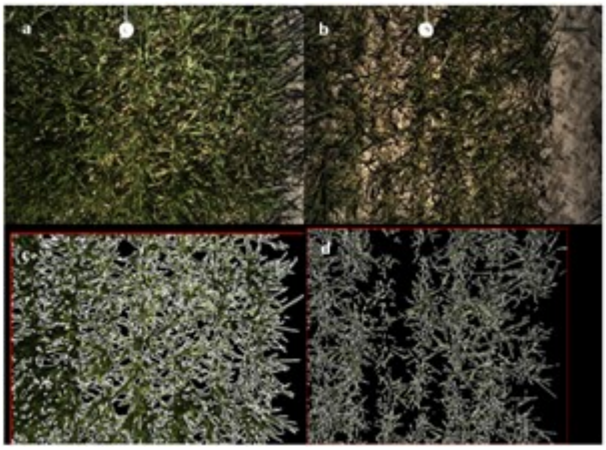

Fig. 5. HTPP of canopy density in spring: original photos (a) well and (b) less dense canopy and the same photos processed using MorphoAnalyser software (c, d).

Table 1

Yield and canopy density in autumn and spring of 12 cultivars of winter triticale subjected to HTPP phenotyping. Grouping based on LSD test with a probability of 95%

Numerical ID	Cultivar	Yield ($t \cdot ha^{-1}$)		Autumn canopy density (a.u.*)		Spring canopy density (a.u.*)	
		Ranking	Mean	Ranking	Mean	Ranking	Mean
6	Octavio	1	10.02	2	1.61	2	53.43
7	Orinoko	2	9.86	4	1.49	8	51.15
1	**Belcanto**	3	9.83	2	1.53	3	53.38
12	Medalion	4	9.65	1	1.62	1	54.60
10	Gringo	5	9.34	7	1.38	4	52.82
4	**Porto**	6	9.29	10	1.22	10	46.45
11	SU Liborius	7	9.12	5	1.39	5	52.76
9	Toro	8	8.91	6	1.39	6	51.64
8	Tadeus	9	8.68	9	1.27	7	51.44
5	Trapero	10	8.02	11	1.22	11	46.13
2	Dolindo	11	7.82	8	1.32	9	49.78
3	**Meloman**	12	7.77	12	1.13	12	41,52
Mean			9.03		1.38		50.42
Controls mean			8.96		1.30		47.12
LSD (5%)			1.58		0.34		8.49
Tukey HSD (5%)			2.77		0.60		14.89
α			0.05		0.11		0.12
Variation coefficient			11.93		16.91		11.48

*a. u. - arbitrary units; 1 a. u. = 0.1 $Mpix \cdot 0.92\ m^{-2}$

field capacity of the soil (soil condition in which water has drained from larger soil pores and is replaced by air), permanent wilting point of plants (soil moisture at which signs of permanent wilting of plants appear), cation exchange capacity (cation exchange capacity) (Skudlarski, 2023). During the visual evaluation of experiments, the breeder records the rating in relative scale units in the field book. At the same time, the HTPP protocol assumes the collection of partial data, which is then evaluated using statistical methods. A field map, built on measurements taken in places from which phenotypic data is collected, is necessary to interpret phenotypic data along with soil quality indicators at the points where the shots were taken.

Yield is the primary determinant of the cultivar's market value. It results from a sum of environmental impacts, and, just like the visual evaluation, is, in a sense, an average value of micro differences conditioned by local differences in the quality of the arable layer. Greater precision in data collection in the HTPP system requires refinement of the methods of averaging them after introducing corrections for a precise location. GPS is needed to build the correct yield model (Rybka and Nita, 2014). This does not diminish the result of the experiment, which shows that automatic HTPP phenotyping can be performed at the plant tillering stage, and the results can be independently analyzed using intuitive software based on building masks of objects and backgrounds. In such a case, it is not necessary to use complex, convolutional neural networks (CNN) (Stefański et al. 2024). Showing this possibility was the aim of this publication. Implementing the HTPP approach into breeding practice requires further work to formulate an effective procedure.

In the experiment, the highest-yielding cultivars were Octavio, Orinoco, Belcanto, and Milan, above 9.5 t·ha^{-1}, and they were also characterized by the highest canopy density, from 1.49 to 1.62 a.u. The reference cultivars in the PDO experiment for winter triticale in the 2019/2020 season were Belcanto, Porto, and Meloman. Their average yield in our experiment was 8.96 t·ha^{-1}, and the canopy density was 1.30 a.u. The following yields were higher than the standard: Octavio, Orinoko, Medalion, Gringo, SU Liborius, and the Toro variety had a canopy density higher than the standard, yielding 99.4% of the standard (Tab. 1).

The level of agrotechnical treatments did not significantly impact the average yield of the tested cultivars, which could be due to the environmental conditions already discussed. Therefore, to obtain clarity in the interpretation of the results for varieties, the A1 and A2 data were averaged for both yield (Fig. 6) and canopy density in autumn (Fig. 7) and spring (Fig. 8). Despite the lack of a statistically significant difference, the A2 level of agrotechnical treatment had a higher yield, which confirms the fact that better protection and more excellent fertilization provide a chance to increase the yield of crops (Drzazga et al., 2013).

Table 2 presents the values of Pearson's correlation coefficients for HTPP, canopy density in autumn and spring, and winter triticale yield and Figure 9 shows the linear charts for those relations. The high correlation coefficient of 0.79 gives hope for the effectiveness of HTPP in phenotyping canopy density, developing the method for other species in Plant Breeding Strzelce, and expanding and improving the high-throughput platform for field observations and building new breeding procedures.

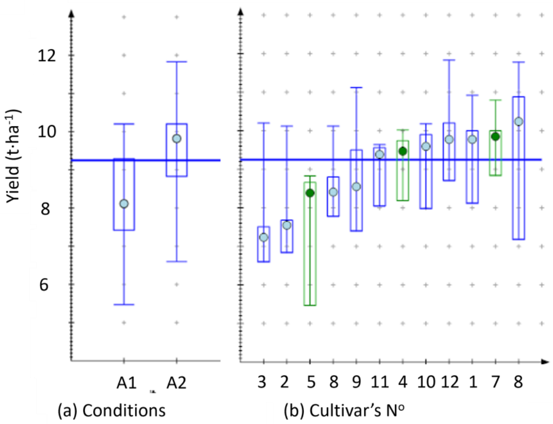

Fig. 6. Boxplot charts for yield depending on: (a) agrotechnical conditions A1 and A2 (b) the tested cultivars. Charts generated by Doriane software.

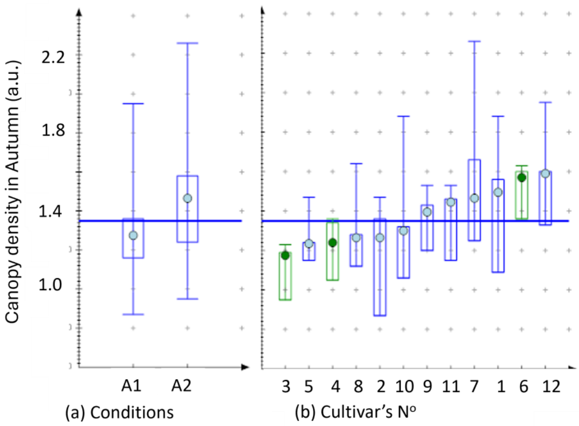

Fig. 7. Boxplot charts for canopy density in the autumn (a) depending on agrotechnical conditions A1 and A2 and (b) the tested cultivares. Charts generated by Doriane software.

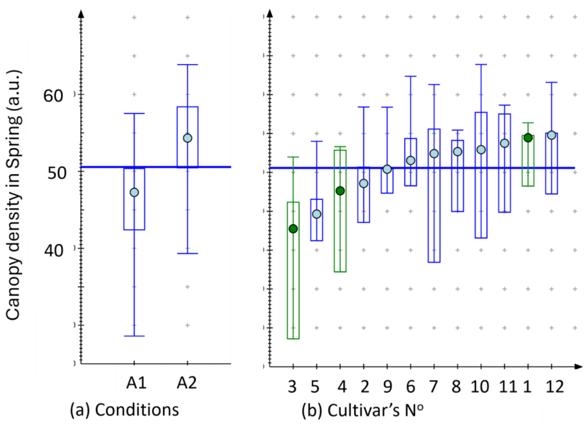

Fig. 8. Boxplot charts for canopy density at spring (a) depending on agrotechnical conditions A1 and A2 and (b) the tested cultivares. Charts generated by Doriane software.

Table 2

Pearson's correlation coefficients between yield and canopy density in autumn and spring, as well as correlations between canopy density in seasons

	Autumn canopy density	Spring canopy density	Yield
Autumn canopy density	1		
Spring canopy density	0.688	1	
Yield	0.794	0.793	1

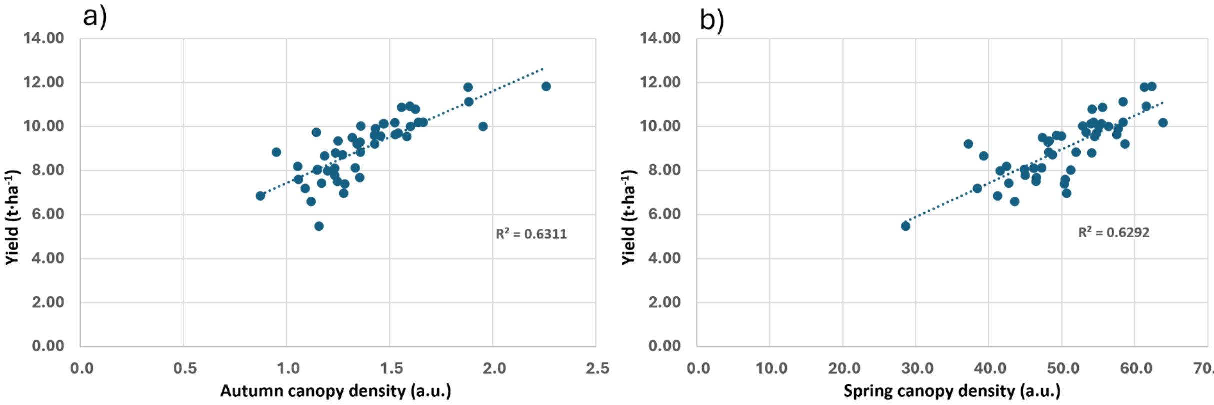

Fig. 9. Relation of winter triticale yield ($t\cdot ha^{-1}$) and canopy density (a) in autumn and (b) in spring. a. u. - arbitrary units; 1 a. u. = 0.1 $Mpix\cdot 0.92\cdot m^{-2}$.

Conclusions

1. High-throughput HTPP phenotyping using different cameras requires new data processing and analysis methods;
   - It should be checked whether the precision of the results can be increased by taking into account correction factors resulted from the soil quality;
2. A strong positive correlation of canopy density assessed using HTPP with yield (0.79) is an important result for breeding and can be implemented as a selection parameter for high-yielding cultivars.

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