



A Case Study on The Evaluation of Maturity Class in Potato Breeding Trials Using UAV Imagery

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Abstract

In potato breeding, maturity class (MC) is a crucial selection criterion because this is a critical aspect of commercial potato production. Currently, the classification of potato genotypes into MCs is done visually, which is time- and labor-consuming. The objective of this research was to use vegetation indices (VIs) derived from unmanned aerial vehicle (UAV) imagery to remotely assign MCs to potato plants grown in trials, representing three different early stages within a multi-year breeding program. The relationships between VIs (GOSAVI - Green Optimized Soil Adjusted Vegetation Index, MCARI2 - Modified Chlorophyll Absorption Index-Improved, NDRE – Normalized Difference Red Edge, NDVI – Normalized Difference Vegetation Index, and OSAVI – Optimized Soil Adjusted Vegetation Index and WDVI – Weighted Difference Vegetation Index) and visual potato canopy status were determined. Further, this study aimed to identify factors that could improve the accuracy (decrease Mean Absolute Error – MAE) of potato MC estimation remotely. Results show that VIs derived from UAV imagery can be effectively used to remotely assign MCs to potato breeding lines, with higher accuracy for the potato B-clones (20 plants per plot) than the A-clones (6 plants per plot). Among the tested VIs, the NDRE allowed for potato MC evaluation with the lowest MAE. Applying NDRE for remote MC estimation using a validation dataset of potato B-clones (100 plants per plot), resulted in an MC estimate with a 0.81 MAE. However, the accuracy of potato MC estimation using UAV image-based methods should be improved by reducing the potato canopy's variability (increasing uniformity) within the plot. This could be achieved by minimizing 1) potato vines bending over the neighboring row, causing vine overlap between plots, and 2) plants damaged by tractor wheels during field operations.

Resumen

En el mejoramiento de la papa, la clase de madurez (CM) es un criterio de selección crucial porque este es un aspecto crítico de la producción comercial de papa. Actualmente, la clasificación de los genotipos de papa en MC se realiza visualmente, lo que requiere mucho tiempo y trabajo. El objetivo de esta investigación fue utilizar índices de vegetación (VIs) derivados de imágenes de vehículos aéreos no tripulados (UAV) para asignar de forma remota MCs a plantas de papa cultivadas en ensayos, representando tres etapas tempranas diferentes dentro de un programa de mejoramiento de varios años. Se determinaron las relaciones entre los VIs (GOSAVI – Índice de Vegetación Ajustado al Suelo Optimizado Verde, MCARI2 – Índice de Absorción de Clorofila Modificado-Mejorado, NDRE – Borde Rojo de Diferencia Normalizada, NDVI – Índice de Vegetación Ajustado al Suelo Optimizado y WDVI – Índice de Vegetación Ajustado al Suelo O

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de Vegetación de Diferencia Ponderada) y la visualización del dosel de la papa. Además, este estudio tuvo como objetivo identificar factores que podrían mejorar la precisión (disminuir el Error Absoluto Medio – MAE) de la estimación de MC de papa de forma remota. Los resultados muestran que los VI derivados de las imágenes de UAV se pueden utilizar de manera efectiva para asignar MC de forma remota a las líneas de mejoramiento de papa, con mayor precisión para los clones B de papa (20 plantas por parcela) que para los clones A (6 plantas por parcela). Entre los VI probados, el NDRE permitió la evaluación de la MC de papa con el MAE más bajo. La aplicación de NDRE para la estimación remota de MC utilizando un conjunto de datos de validación de clones B de papa (100 plantas por parcela), resultó en una estimación de MC con un MAE de 0.81. Sin embargo, la precisión de la estimación de la MC de la papa (umentando la uniformidad) dentro de la parcela. Esto podría lograrse minimizando 1) los tallos de papa que se doblan sobre el surco vecino, lo que causa la superposición de follaje entre las parcelas, y 2) las plantas dañadas por las ruedas de los tractores durante las operaciones de campo.

Keywords Potato breeding \cdot Crop phenomics \cdot Maturity class \cdot Digital agriculture \cdot Unmanned aerial vehicle \cdot UAV \cdot Imagery \cdot Vegetation index

Introduction

Potato (Solanum tuberosum L.) is a globally significant food crop, ranking as the third most important after rice and wheat and the foremost vegetable crop, producing 375 million tons in 2022 (FAOSTAT 2024). Conventional plant breeding, based on the hybridization of parents and phenotypic selection of offspring, while very effective, is also slow (Spindel and McCouch 2016). Reducing the time required to shrink the population size improves the economic feasibility of the breeding program. Among the about seventy traits the potato varieties are screened for (Zimnoch-Guzowska 2017), there is also a maturity class (MC), that expresses the length of the vegetation period required to produce a harvestable product (Plich 2017), a critical aspect of commercial potato production. Maturity is a complex phenomenon affected by many potato growth and development components (Khan et al. 2013). However, the variation in maturity between the genotypes could be reflected in differing periods of attaining critical physiological stages such as canopy development, tuber initiation, filling, and total duration. Currently, the classification of potato breeding lines and varieties into MCs is still done visually on thousands of experimental plots by an experienced breeder who monitors the vine characteristics at particular time intervals during the crop cycle (Haga et al. 2012). To maintain the same classification standard throughout the germplasm nursery, the same person must perform the procedure quickly. This time- and labor-consuming task can be additionally limited by unfavorable ground conditions such as rainy weather, associated high soil moisture, and changing light conditions that affect the human perception of the plant color. Remote estimation of potato MC can partially overcome these limitations because flights with unmanned aerial vehicles (UAVs) can be done at times of high soil moisture and at changing light conditions, providing that radiometric calibration of the image is performed if comparing between collection dates (Aasen et al. 2018). Moreover, the UAV can be flown over the potato experiments, covering several thousand small plots, more often than the visual observation done by the breeder, and used to evaluate multiple plant traits other than the MC alone. The high temporal resolution of data collection with UAVs allows for timely estimation of the essential plant traits rather than missing the critical stages in plant development (Burkart et al. 2018). This approach reduces input costs, saves time, and minimizes errors caused by multiple rounds of phenotyping done by various people when evaluating the same plots (Chawade et al. 2019).

Recently, UAVs have been used for various aspects of potato plant phenotyping. The work by ten Harkel et al. (2020) in the Netherlands has shown that data derived from LiDAR (Light Detection and Ranging) mounted on the UAV could estimate fresh potato biomass and plant height. However, the accuracy of estimation of both plant traits was less reliable (compared to other crops like sugar beet and wheat) due to the complexity of plant canopy field architecture because potatoes are grown in beds. Li et al. (2020) selected crop heights derived from UAV imagery and vegetation indices (VIs): CI1 - red-edge chlorophyll index 1; MSR - modified simple ratio as the best potato yield and biomass predictors. Those researchers stated that crop heights obtained in an automated way are likely to be more accurate than manually estimated crop heights from limited sampling. The study by Colwell et al. (2021) demonstrated how the use of point cloud data obtained from low-cost UAV imaging can be used to create 3D surface models of the plant canopy, from which detailed and accurate data on plant height and its distribution, canopy ground cover and canopy volume can be obtained over the growing season. Franceschini et al. (2017) compared ground-based and UAV-mounted spectrometers in organic potato cultivation. The UAV-based canopy structure and leaf chlorophyll estimates, leaf area index (LAI), and ground cover were relatively more accurate than those derived from ground-based measurements. Recently, Matias et al. (2020) developed the FIELDimageR package, which includes functions allowing the determination of the number of plants per plot, canopy cover percentage, VIs, and plant height from UAV orthomosaic images of potato field trials. However, there are still gaps in the literature regarding the application of vegetation indices derived from UAV orthomosaics for potato MC estimation in breeding trials.

The objectives of this study were to 1) establish the relationship between VIs developed using UAV-derived spectral data (remote method) and visual potato canopy status estimation (vine greenness and growth habit) and determine the effectiveness of these measurements in detecting differences among MCs at two stages of the potato breeding program; 2) validate the remote MC evaluation using a set of new potato genotypes grown as a third

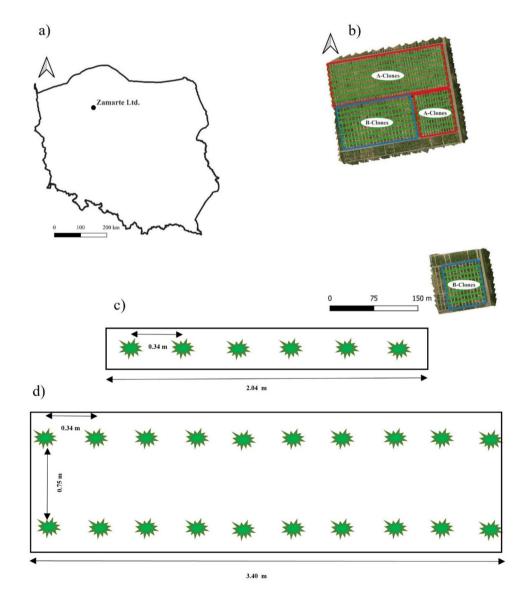
stage of the potato breeding program; and 3) identify factors that could improve the accuracy of remote potato MC estimation.

Methods

Experimental Sites and Growing Conditions

Research was conducted in 2021 on regular potato breeding trials of the Zamarte Potato Breeding Ltd. company at Zamarte in northern Poland ($53^{\circ}36'9.22"N,17^{\circ}29'16.05"E$, elevation: 148 m) (Fig. 1a). The trials covered 4660 plots with potato A-clones (breeding lines A – second field generation/propagation which consists of all potato genotypes tested at the earlier stage of the breeding program, and 1320 plots with potato B-clones (breeding lines B – third

Fig. 1 Illustration of experiments set up (a) location of breeding trials of the Zamarte Potato Breeding Ltd. company at Zamarte, Poland, in 2021, (b) overview of the location of the trials with potato A-clones (parts 1 and 2, red outlines) and B- clones (parts 1 and 2, blue outlines) superimposed on the UAV images taken on July 25 and 26, 2021. Green strips represent the location of the standard potato varieties. Scheme of a single potato plot of (c) A-clones and (d) B-clones



field generation/propagation) - the later stage of the breeding program (Fig. 1b). UAV imagery was used at these two stages of the breeding program because the classification of potato genotypes into MCs is done at this time visually to the highest number of plots, which is time- and labor-consuming. Each plot consisted of one row (bed) of 6 plants and two rows of 10 plants each for the A- and B-clones, respectively (Fig. 1c and 1d). A set of standard potato varieties: Bielik (1), Impresja (1), Gala (3), Ismena (3), Longina (3), Madeleine (5), and Oberon (5) in Aclones experiment and Werbena (1), Bielik, Gala, Ismena, Longina, Madeleine, Oberon and Widawa (5) in B- clones experiment, covering a wide range of MCs (given in the brackets) under Polish conditions was planted to benchmark the effects of maturity type among known genotypes. All outer beds of the experiments were planted with a very early variety of Milek (1). The spacing between plants was 0.34 m, and the total row length was ~ 2.04 m for the Aclones and ~3.40 m for the B- clones (Fig. 1c and 1d). The bed width was 0.75 m. To eliminate the border effect, there was no distance between plots. Potatoes were hand planted for A- clones experiments on April 21, 2021, and for B-clones experiments on April 23 and 28, 2021. All field operations were conducted by technical staff of the Zamarte Potato Breeding Ltd. company. The potato A-and B- clones breeding trials were harvested on August 25–26, 2021, and September 1 and 2, 2021, respectively.

The research location is characterized by a moderate climate with the year average air temperature of 8.1 °C and cumulative participation of 612.4 mm for the 30-year period of 1991-2020 registered by a weather station of the Institute of Meteorology and Water Management (IMWM 2024) placed in Chojnice c. 10 km from the research trials (https:// klimat.imgw.pl/pl/climate-normals/TSR_AVE). The soils of the trials corresponded mainly to Luvisols and Cambisols (IUSS Working Group WRB 2014), with loamy sand in the plow layer. Rainfall was registered by a manual Hellmann rain gauge of 200 cm² surface area (P.P.U.H WOBIS, Zabrze, Poland), located near the potato trials. Air temperatures were obtained from the IMWM weather station. The average monthly mean, maximum, and minimum air temperatures during the study period (April-August 2021) were 16.5, 21.7, and 11.5 °C, respectively (Fig. 2). The total rainfall for that period was 181 mm, with half of this amount registered in April-May, and the other half in July-August. According to Chmura et al. (2013), this amount of rainfall on medium to light soils in Polish conditions was suboptimal for growing potatoes. This is because to achieve the maximal vield, rainfall distribution should be about 143 mm during May-June and 220 mm in July-August. Thus, the experiment was carried out in a year with insufficient rain in most of the growing season.

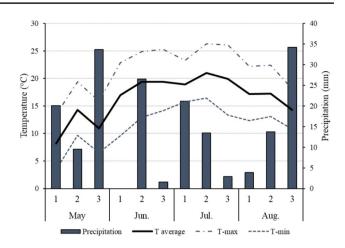


Fig. 2 Monthly averages of maximum, minimum, and mean air temperatures and precipitation from May to September 2021. Numbers 1, 2, and 3 on the X-axis refer to the successive 10 or 11-day average to present the distribution of precipitation within each month

The validation trial with potato C-clones (breeding lines C), located at Zamarte $(53^{\circ}35'31"N, 17^{\circ}30'08"E,$ elevation: 148 m) ~ 730 m south from the B-clones (part 2) trial, was the fourth field generation/propagation of the breeding program after the potato B-clones (Fig. 1b). This experiment included 240 out of the 4660 potato genotypes that were previously grown in 2021 as potato A-clones. Potatoes were hand planted on April 25, 2023. The single plot covered an area of 25.5 m² and consisted of two beds (like in the trial with the potato B-clones) but with 50 plants grown in each of the beds. Spacing between plants was 0.34 m, the bed width was 0.75 m, and the total row length was 17 m (Fig. 1c and 1d).

Visual Observations

The visual estimation of the MC of potato A-clones and B-clones, based on a genotype plant growth habit, uniformity of growth, and intrinsic vine color, was performed during intensive yellowing and bending of potato vines over the beds of very early standard varieties: *Bielik* and *Impresja*. The MC estimation was done according to the Research Centre for Cultivar Testing (2018) on a 9-degree scale, namely:

- 1 very early.
- 2 very early to early.
- 3 early.
- 4 early to medium.
- 5 medium.
- 6 medium to late.
- 7 medium late.
- 8 late to very late.
- 9 late.

The visual estimation of the MC of potato A-clones and B-clones was performed by an experienced breeder, respectively, on July 22 and 23 and July 26 and 27, 2021. Among the potato A and B-clones tested in the Zamarte Potato Breeding Ltd. breeding trials, the MCs range from 1 to 7. Very early A-clones, with a completely dry canopy during the visual estimation, were additionally assigned an MC of 0.5.

Commercial standard (reference) varieties, planted as a benchmark against the MC of the tested A and B-clones, representing various plant MC (given in Sect. 2.1) MC, namely *Bielik, Impresja, Gala, Ismena, Longina, Madeleine,* and *Oberon*, were planted every ten plots in the A-clones trial. *Werbena, Bielik, Gala, Ismena, Longina, Madeleine, Oberon* and *Widawa* varieties were planted in every five plots in the B-clones trial (Fig. 1b).

Between June 14 and July 2, 2021, a negative selection was performed twice to remove clones with a virus, infection of blackleg (*Pectobacterium atrosepticum* and *Dickeya spp.*, van Hall; Robert S. Dickey), and rogues (i.e., those plants that differed from other plants of the exact clone). In total, 3883 A-clones and 1059 B-clones were assigned the MC and used for statistical analysis. Visual estimation of the MC in the validation trial with C-clones was performed on July 28, 2023. The negative selection in this trial was performed on June 22 and 23, 2023. In total, 120 new potato genotypes were assigned an MC and used for statistical analysis to validate the usefulness of the VIs for remote MC estimation.

UAV Image Acquisition

The ariel-based data was collected by UAV (Phantom 4 Multispectral, DJI, Shenzhen Dajiang Baiwang Technology Co., Ltd., China), equipped with a highly accurate Global Navigation Satellite System with RTK (Real Time Kinematic) correction. Flights were conducted on July 25 and 26, 2021, perpendicularly to sunlight direction close to solar noon, on cloud-free days, to avoid changing light conditions. The UAV speed was about 1 m/s, flown 20 m

Table 1 Summary of vegetation indices

above ground level to achieve high spatial resolution, resulting in a 1.1 cm pixel⁻¹ image spatial resolution. UAV images were taken by DJI's multispectral FC6360 camera with 80% front and side overlap. The Phantom 4 Multispectral imaging system contains six cameras with 1/2.9" CMOS sensors, including an RGB camera and a multispectral camera array of the following bands: blue (B) $-450 \text{ nm} \pm 16 \text{ nm}$, green (G) 560 nm \pm 16 nm, red (R) – 650 nm \pm 16 nm, red edge (RE) -730 nm ± 16 nm, and near-infrared (NIR) $-840 \text{ nm} \pm 26 \text{ nm}$. The spectral sunlight sensor on top of the aircraft detects solar irradiance in real time for image compensation, maximizing the accuracy of collected multispectral data. Images of the validation trial with the C-clones were taken on July 26, 2023, using the same UAV platform and flight settings as for the potato A and B-clones trials. The radiometric calibration to the images was not applied in 2021 but was applied in 2023.

UAV Image Processing

The multispectral images were uploaded to the Pix4Dmapper computer software (Pix4D S.A. Prilly, Switzerland) for processing and generating orthorectified reflectance maps. Five reflectance maps were generated, one for each spectral band (B, G, R, RE and NIR). Using OGIS.org (2022), plot shape files were created and manually placed over each of the single potato plots (Fig. 1c and 1d). The image reflectance maps and shape files were exported to ENVI (Harris Geospatial Solutions Inc. Broomfield, CO, United States) for data analysis. The five reflectance datasets were combined to create a composite-band stack. The plot shape files were converted into a rectangular region of interest (ROI) of 0.60 (width) by 2.04 m, 1.2 (width) by 3.40 m, and 1.2 (width) by 17 m for the plots of the A-clones, B-clones, and the C-clones, respectively. The width of the frame was narrower than the bed width of 0.75 m and the double width of 1.50 m for the potato A-clones and B-clones, respectively. This was done to avoid deviation of the VIs for the plot regions where the plants from the neighboring plots might have overlapped. A total of six

Vegetation Index	Abbreviations	Formula	Reference
Green Optimized Soil Adjusted Vegetation Index	GOSAVI	$(\rho NIR - \rho G) / (\rho NIR + \rho G + 0.16)$	Sripada et al. 2005
Modified Chlorophyll Absorption Index-Improved	MCARI2	$\frac{1.5*[2.5(\rho NIR-\rho R)-1.3(\rho NIR-\rho G)]}{[(2*\rho NIR+1)^2-(6*\rho NIR-5*\rho R^{1/2})-0.5]^{1/2}}$	Haboudane et al. 2004
Normalized Difference Red Edge	NDRE	$(\rho NIR - \rho RE)/(\rho NIR + \rho RE)$	Barnes et al. 2000
Normalized Difference Vegetation Index	NDVI	$(\rho NIR - \rho R)/(\rho NIR + \rho R)$	Rouse et al. 1974
Optimized Soil Adjusted Vegetation Index	OSAVI	$(\rho NIR - \rho R)/(\rho NIR + \rho R + 0.16)$	Rondeaux et al. 1996
Weighted Difference Vegetation Index	WDVI	$\rho NIR_c - (\rho NIR_s / \rho G_s) * \rho G_c$	Evert et al. 2012

Abbreviations: NIR: near-infrared; RE: red-edge; R: red; G: green

 ρ :reflectance; c: reflectance of the crop canopy; s: reflectance of the bare soil

VIs were calculated for all datasets (Table 1). We chose these specific VIs to use the bands registered by the DJI's multispectral FC6360 camera for their calculation and to rely on VIs sensitive to various vegetation parameters. To compensate for soil background influences, the GOSAVI - Green Optimized Soil Adjusted Vegetation Index and OSAVI - Optimized Soil Adjusted Vegetation Index were used (Rondeaux et al. 1996; Sripada et al. 2005). The MCARI2 (Modified Chlorophyll Absorption Index-Improved) was used because it was proved by Haboudane et al. (2004) to be an excellent predictor of green leaf area index, which represents living leaves regardless of their photosynthetic capacity. The WDVI (Weighted Difference Vegetation Index) was calculated because it correlated well with potato nitrogen uptake (van Evert et al. 2012). Additionally, NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Red-Edge), commonly used for monitoring of crop canopy but not requiring sophisticated calculations, were also applied (Rouse et al. 1974; Barnes et al. 2000). The MCARI2, NDVI, GOSAVI and OSAVI were automatically generated using the ENVI software's built-in functions. There are no such functions to generate NDRE and WDVI. Therefore, the NDRE was manually generated by adding the equation and assigning the respective bands to each variable using the Band Math function in ENVI. For WDVI, the calculation was more complex due to the specific requirements in utilizing a combination of bands (green and NIR) and segregated canopy and soil reflectance values. To obtain the segregated values, the average soil reflectance for the green and NIR bands was calculated by creating a supervised classification of the original image consisting of the crop, shadow, and soil (Fig. 3). The classified image was converted into a binary image where the pixels corresponding to the crop and shadow have no value, while the pixels corresponding to the soil were assigned a value of one (Fig. 3c). The binary image was then applied to the original reflectance maps, resulting in an image dataset consisting of only the soil pixels with their respective pixel values (Fig. 3e). Then the pixel values corresponding to the soil and shadow were converted to have no value. The binary mask was applied to the original reflectance maps to generate the dataset with only the canopy pixels and

respective pixel values (Fig. 3b and 3d). The average soil and canopy reflectance was extracted for the green and NIR bands and used to compute the WDVI (Fig. 3f). After all the VIs were computed, a new band stack was created that included all VIs. The ROI was placed over the VI band stack, and pixel statistics were generated for all plots. The pixel statistics text file was then exported to a spreadsheet for data filtering and further statistical analysis.

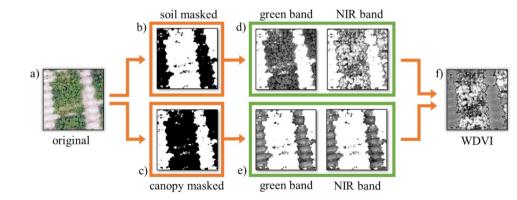
On-the-Ground Image Collection

Potato plant overhead images were captured by a camera builtin in a Mi 10 T-Lite Xiaomi (Xiaomi Communications Co., Ltd., Beijing, China) cell phone facing downwards at a distance of ~ 1.2 m above the ground, with a pixel size of 3472×4624 . Images were only taken for plots with specific plant appearance, e.g., potato vines bending on the neighboring rows or between them in furrows, damaged plots, clones of distinguished color, and visible weed infestation. This approach of taking ground images helps to have a close look at the details of plant appearance that cannot be noted using UAV images and breeder descriptions. Moreover, the on-the-ground photos help to demonstrate confounding environmental factors.

Statistical Analysis

Statistical metrics to assess the accuracy of the predicted MC based on remotely evaluated VI were calculated using Microsoft® Excel (Microsoft Corporation, Redmond, Washington, United States). Pearson's correlation coefficient (r), mean absolute error (MAE), and percent coefficient of variation (CV) were used to measure the linear correlation, error, and dispersion between the MC and VI, respectively. Correlations at $\alpha = 0.05$ were considered significant. The top VIs were determined based on the highest correlation with the MC and lowest MAE and CV values. Additionally, linear regression was computed to assess the strength of the relationship between the top VIs and MC based on the coefficient of determination (R²). The top VI was used to estimate the MC on the validation data set using the linear regression equation.

Fig. 3 The workflow of creating the WDVI, starting with the original image (**a**); converting the soil and canopy to separate binary masks (**b** and **c**); applying the masks to the green and NIR bands to generate the canopy only (**d**) and the soil only (**e**); then generating the WDVI (**f**)



Results and Discussion

Distribution of Maturity Class Among the Potato A-clones and B-clones

The number of plots without MC assigned was 777 (16.7%) and 261 (19.8%) for the potato A-clones and B-clones trials, respectively. These plots were excluded during the negative selection.

The highest number, among the total 3883 – A-clones of plots, was found for the MC of 3 (41.6%) and MC of 5 (33.0%) (Fig. 4a). Ninety-five plots (2.45%) were assigned an MC of 0.5. These very early maturing forms (with a very short growing season) are desirable in breeding because potato production for early harvest is the most profitable in Poland, and therefore, breeding objectives at Zamarte Potato Breeding Ltd. are focused on such table cultivars (Kamiński 2017). Among the total 1059 B-clones visually estimated, the highest number of plots was assigned to the MC of 3 (24.4%) and MCs of 4 and 5, each 19.6% (Fig. 4b).

The distribution of the MC among the potato A-clones and B-clones should not be compared because these genotypes come from different crossing combinations (breeding programs established in different years), resulting in various frequencies of MCs. However, such comparisons are acceptable from a technical point of view, i.e., to verify if there are significant differences (related to the degrees of freedom) in the relationship between VIs derived from UAV imagery and visually estimated MC between potato A-clones and B-clones grown in plots of 6 and 20 plants, respectively.

At the earlier stage of the potato breeding program, the range of genotypes grown is much more comprehensive (MC from 0.5 to 7) because only the best-performing genotypes among the potato A-clones are selected at the later stage of the program (B-clones). For example, of the 4660 genotypes grown in the potato A-clones in 2021, only 1392 were selected as the B-clones to be grown in 2022. Consequently, in 2023 experiment with potato C-clones included 240 genotypes out of the 1392 potato genotypes that were grown in

2021 as potato B-clones. Tracking by a breeder of the MC distribution among the potato genotypes in the subsequent generations of the breeding program, supported or replaced by the use of UAV imagery, would enhance potato breeding. The main goal of the breeding process of the Zamarte Potato Breeding Ltd. company is to produce very early and early potato varieties, but late-maturing starch varieties (to extend the period of this carbohydrate accumulation in their tubers) in very limited numbers are also bred. Therefore, the much more normal distribution of the MC in the potato B-clones population compared to the potato A-clones is accidental.

Changes in Vegetation Index Values Across the Maturity Classes of Potato A-clones and B-clones

To calculate the vegetation index values across the maturity classes, the VI values from each MC were averaged. In the case of the B-clones, the values of all VIs increased with MC (Fig. 5b). In contrast, values of all VIs for the A-clones increased up to the MC of 4 and then showed a tendency to increase for the MC of 5 to 7 (Fig. 5a), the MC of 6 was characterized by the highest values of all VIs. The non-smoothness (two peaks in Fig. 5a) of the A-clone curve likely reflects the low number of plots in MC groups 4 and 6 in the A-type clones (Fig. 4a). But on the other hand, it is also plausible that the breeder assigned an overly elevated MC of 4 and 6 to specific plots as the natural assumption is that higher MC (later maturing) is positively correlated with denser and greener potato canopies. This result may show that it is more challenging to correctly assign MC to a plot with a denser canopy of one row of the A-clones plants than a plot with a denser canopy of the two-row plants of the B-clones plots. Namely, a plot with 20 plants of the B-Clone is more representative of the MC than a plot with six plants of the A-Clone. The VIs used for MC evaluation do not provide direct information on canopy density, but the NIR band often tends to increase linearly with increasing biomass. This is because the NIR canopy reflectance is a function of scattering within the mesophyll layer of plant leaves and higher leaf

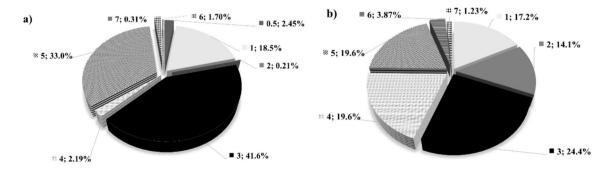


Fig. 4 Distribution (%) of maturity class among potato a) A-clones (numbers from 0.5 to 7) and b) B-clones (numbers from 1 to 7)

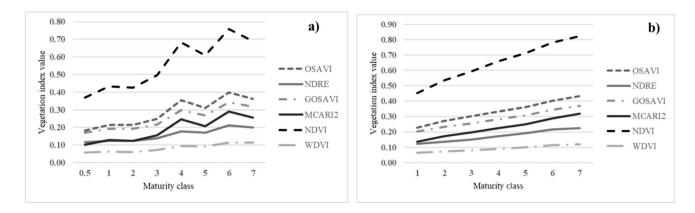


Fig. 5 Changes of vegetation index values across the maturity classes of the potato: a) A-clones and b) B-clones

transmittance (Holland et al. 2012), thus strongly dependent on the structural properties of the canopy.

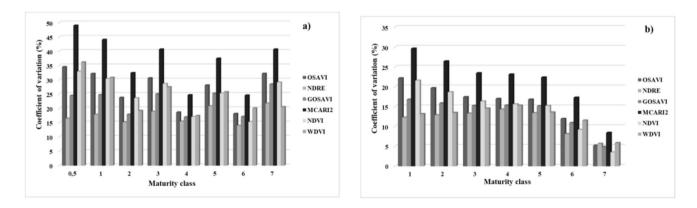
According to Colwell et al. (2021), the breeders' bias and inaccuracy apply not only to commercial potato traits (e.g., yield, tuber shape, and size) but also to traits strongly associated with plant performance (e.g., canopy development and architecture, and growth rates).

Variability of Vegetation Indices Values Between the Maturity Classes of Potato A-clones and B-clones

Among all the VIs tested in both potato trials, NDRE and GOSAVI showed the lowest variation within MCs of A-clones and NDRE and WDVI within MCs of B-clones (Fig. 6a and 6b). However, there were minimal differences between WDVI and GOSAVI regarding A-clones. In comparison, MCARI2 had the highest variation in all MCs (Fig. 6a and 6b). According to Haboudane et al. (2004), MCARI2 is less sensitive to chlorophyll concentration variations (foliage cover with various pigment contents), which is the primary factor that influences LAI retrieval from reflectance data. This observation means that NDRE, GOS-AVI, and WDVI are more useful for the remote evaluation of the potato maturity class because a smaller number of differently-looking plots is assigned to the same MC.

The number of potato plots in each MC differed (Fig. 4a and 4b), so the MC with the highest number of plots could have had the highest variability of the VIs. However, as expected, the CV of VI values did not increase with the number of potato plots in each MC. In the case of the B-clones (Fig. 6b), the CV of all VI values showed an apparent tendency to decrease with the increasing number of the MC. A similar but less clear tendency was observed for the A-clones. The much lower CV values of the VI for the MC with a higher number suggest that potato plots within one of these MCs looked similar in biomass and color. This is because spectral indices that use combinations of red (Red) and near-infrared (NIR) reflectance are potential candidates for estimating the quantity and quality of green vegetation (Holland et al. 2012).

Relationship Between the Maturity Class and Vegetation index Values for Potato A and B-clones



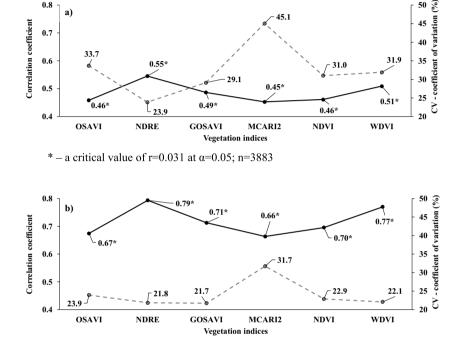
Potato varieties are characterized by specific physiological differences, such as leaf shape and color, or biochemical

Fig. 6 Coefficient of variation (%) of the vegetation index values within the maturity classes of potato: a) A-clones and b) B-clones

differences that affect potato spectral responses, as Gold et al. (2019) observed. All tested VIs showed a significant relationship with the MC assigned by the breeder to Aand B-clones (Fig. 7a and 7b). Among all the compared VIs for the A- and B-clones, NDRE, and WDVI had the strongest relationship with the MC assigned. The correlation coefficient values (r) were 0.55 and 0.51 for NDRE and WDVI for the A-clones and 0.79 and 0.77 for the B-clones, respectively. Therefore, a scatter plot of the VIs values versus maturity class showing the highest correlation values

Fig. 7 The correlation coefficient (r) values (solid line) for the relationship between the maturity class and vegetation indices values; and coefficient of variation (%) of vegetation indices values (grey, dotted line) within the vegetation indices for (a) potato A-clones and (b) potato B-clones (NDRE and WDVI) expressed by R^2 among the VIs is presented in Fig. 8.

The higher values of the correlation coefficient for the B-clones indicate that the MC can be remotely evaluated with higher precision for the plots with the two-row plants of the B-clones. However, the NDRE values were generated using the ENVI software using only the Band Math function in ENVI, but WDVI was calculated using the Band Math function plus additional image processing to isolate the soil and green canopy in ENVI. Therefore, the NDRE could be



* – a critical value of r=0.06 at α =0.05; n=1059

Fig. 8 A scatter plot of the vegetation indices values versus maturity class for (**a**) potato A-clones and NDRE; (**b**) potato A-clones and WDVI; (**c**) potato B-clones and NDRE; (**d**) potato B-clones and WDVI, including R² values

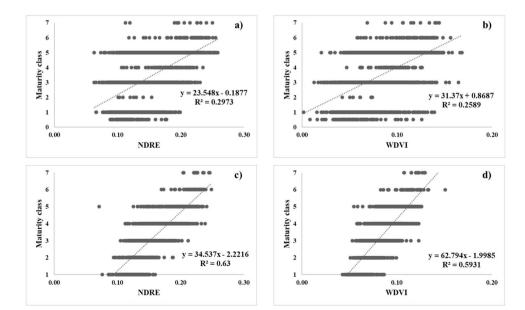
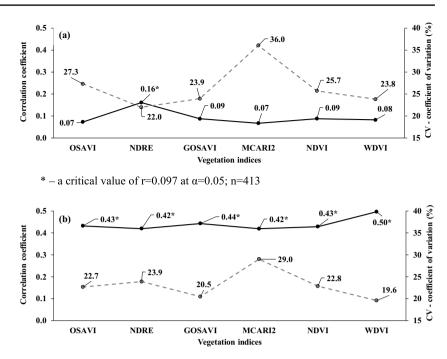


Fig. 9 The correlation coefficient (r) values (solid line) for the relationship between the maturity class and vegetation indices values and coefficient of variation (%) of vegetation indices values (grey, dotted line) within the vegetation indices for potato standard varieties grown in the potato (**a**) A-clones trials and (**b**) potato B-clones trials



* – a critical value of r=0.129 at α =0.05; n=231

preferred for the remote evaluation of the MC in both potato breeding trials due to the less time-consuming procedure.

Relationship Between the Maturity Class and Vegetation Index Values for Potato Standard Varieties

Potato standard varieties grown in the potato A and B-clones trials as the reference varieties (Fig. 1b) of known MC were characterized by a weaker relationship between all tested VIs and MC assigned (Fig. 9a and 9b) than genotypes grown in the potato A and B-clones (Fig. 7a and 7b). The main reason for this weaker relationship could be related to the low number of MCs (only three: 1, 3, and 5), assigned to the standard varieties versus MCs from 0.5 to 7 in the potato A clones trial (Fig. 4a), and MCs from 1 to 7 in the potato B-clones trial (Fig. 4b), but the variation of the VIs values for each VI characterizing these varieties was high (Fig. 9a and 9b) and comparable to the variation of the VIs values in the potato A and B-clones trials (Fig. 7a and 7b). The level at which the relationship between all tested VIs and MC assigned was statistically significant cannot be directly compared for the potato standard varieties and genotypes grown as the potato A and B-clones. This was because the number of plots was much higher in the case of the potato genotypes grown as the A and B-clones than in the case of the standard varieties (see critical values of r in Fig. 7 and 9).

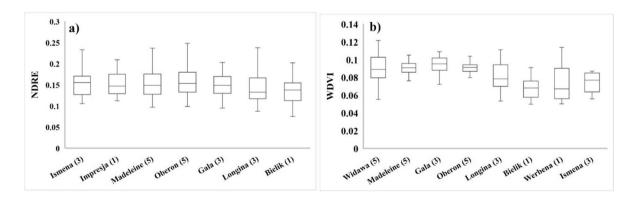


Fig. 10 Variation of NDRE and WDVI values for each potato standard variety grown in the potato (a) A-clones trials and (b) potato B-clones trials, respectively. The number in a bracket refers to a maturity class originally assigned to that standard potato variety

Among all the compared VIs for the potato standard varieties, NDRE (r=0.16) and WDVI (r=0.50) showed the strongest, significant relationship with the MC assigned, respectively, in the A and B-clones trials. The correlation coefficient values for the relationship between the MC and VIs are substantially lower for the potato standard varieties than for the genotypes grown in the potato A and B-clones trials (Fig. 7 and 9). This results from a wide range of VIs (e.g., NDRE and WDVI) values observed for a single standard variety assigned to one MC (Fig. 10a and 10b). Moreover, the range of NDRE values for standard varieties cropped in the A-Clone trial, representing different MCs overlapped, e.g., variety *Impresja* (MC of 1) and *Oberon* (MC of 5). The same was found for WDVI values for standard varieties grown in the A-Clone trial: *Werbena* (MC of 1) versus *Widawa* (MC of 5).

This is a natural consequence of standard varieties being assigned fixed MC values, developed at the stage of their testing before registration in the Common catalogue of varieties of agricultural plant (CCA 2023), and not adjusted to year- and site-specific conditions. However, the potato canopy of the same variety grown in many replications within the A and B-clones trials, evaluated remotely with the use of the VIs, differs probably due to soil spatial variability. The standard potato varieties grown in the two-row plots (20 plants per plot) among the B-clones showed a higher correlation between the MC and VIs values than those grown in the single-row plots (6 plants per plot). This confirms the observation made for the genotypes tested in the trials with the potato A-clones and B-clones that a plot with 20 plants is more representative of the MC than a plot with six plants. Because the range of MC and VIs values is much narrower for the potato standard varieties than for the A and B-clones, the use of the database of the potato standard varieties to improve the accuracy of potato MC estimation remotely seems limited. However, the standard varieties of known MC might still be used as a reference while evaluating the MC of the A- and B-clones potato genotypes.

The MAE of the Maturity Class of Potato Aand B-clones, Predicted Based on Vegetation Index Values

Results showed that NDRE and WDVI were characterized by the lowest MAE values of 1.03 and 1.05 for the potato A-clones and 0.73 and 0.78 for the potato B-clones, respectively (Table 2). These MAE values indicate that, on

Table 2 The MAE of the maturity class of potato A-clones and B-clones is predicted based on vegetation index values

Measures of MC prediction error	Potato A-clones							
	OSAVI	NDRE	GOSAVI	MCARI2	NDVI	WDVI		
MAE	1.11	1.03	1.08	1.10	1.11	1.05		
Percentage of plots with an absolute residual value of 1 or less	50.8	54.6	51.8	50.9	50.9	53.2		
The maximum value of an absolute residual	4.88	4.54	4.82	4.77	4.90	4.32		
Measures of MC prediction error	Potato B-clones							
	0.90	0.73	0.85	0.91	0.87	0.78		
Percentage of plots with an absolute residual value of 1 or less	62.1	72.3	64.7	61.4	63.6	69.7		
The maximum value of an absolute residual	5.41	4.76	5.29	4.83	5.70	3.57		

 Table 3
 The percentage of plots with a different absolute residual value of the maturity class, calculated based on NDRE, within a total number of plots with a particular maturity class for potato A-clones

Range of the absolute	Maturity class								
residual value	0.5	1	2	3	4	5	6	7	
≥0.50	0	0.28	37.5	59.2	58.8	22.2	7.58	0.0	
0.51-1.0	0	4.31	37.5	30.6	28.2	19.1	43.9	0.0	
1.01-1.50	7.37	31.0	25.0	7.67	10.6	19.3	21.2	8.33	
1.51-2.0	45.3	37.6	0	2.17	2.35	21.9	16.7	33.3	
2.01-2.5	32.6	17.2	0	0.37	0	13.5	3.03	16.7	
2.51-3.0	9.47	7.22	0	0	0	3.36	4.55	25.0	
3.01-3.5	5.26	2.36	0	0	0	0.55	3.03	0.0	
<3.5	0	0	0	0	0	0.08	0	16.7	

average, MC could have been evaluated in the case of the A-clones with an error close to one MC and an error of about 0.75 MC for the B-clones. These values of MAE for the MC remote evaluation may seem too high from the breeder's perspective. Therefore, we calculated the percentage of plots for which the MC was assigned with a value of the mean absolute error of less than 1. Because the MAE values were the lowest for NDRE, the highest number with an MAE value of less than one was achieved for the same VI. 54.6% of the A-clones and 72.3% of B-clones were assigned the MC with the MAE value of less than 1.

For single potato plots of the A- and B-clones, the MC was assigned with a very high MAE of 4.54 and 4.76, respectively. This was probably a potato genotype with a stem-type canopy architecture and, therefore, characterized by low VI values derived from UAV images but assigned by the breeder due to its still relatively high greenness as a high MC genotype. The second reason for the very high MAE could have been the breeder's accidental assignment of a high MC value to a potato plot with very low biomass. Among the standard potato varieties are genotypes of different canopy types. For example, among the very early varieties, Bielik is characterized by a stem-type canopy, while

Werbena is a leafy-type canopy variety. Consequently, no clearly visible relations exist between the MC and canopy type among the A- and B-clones.

Percentage of Plots with A Different Absolute **Residual Value of the Maturity Class**

The percentage of plots with a different absolute residual value of the maturity class (Tables 3 and 4) was calculated based on NDRE because this VI was characterized by the lowest MAE (Table 2).

Maturity classes 2, 3, and 4 were calculated using NDRE with the lowest MAE. This is because 37.5%, 59.2%, and 58.8 of the plots, respectively, with the MCs 2, 3, and 4, were assigned to the MC with the absolute residual value of ≥ 0.50 (Table 2). The percentage of plots for the same MCs assigned with the absolute residual value between 0.51 and 1.0 was 37.5, 30.6, and 28.2, respectively. Regarding the other MCs, the maturity class was more often assigned with an absolute residual value of above 1.50. This means that in the case of potato A-clones, it was easier to assign a proper MC using NDRE when the plants were classified to the medium MC of 3, 4, and 5 and then to MCs representing

Danga af tha abaaluta	Maturity class							
Range of the absolute residual value	1	2	3	4	5	6	7	
≥0.50	16.5	51.7	53.5	39.9	44.2	41.5	0	
0.51-1.0	40.1	26.2	31.0	32.7	26.9	31.7	15.4	
1.01-1.50	28.6	20.1	13.2	18.8	14.4	14.6	46.2	
1.51-2.0	12.6	0.67	1.94	6.25	5.77	7.32	23.1	
2.01-2.5	2.20	1.34	0.39	2.40	6.73	4.88	15.4	
2.51-3.0	0	0	0	0	1.44	0	0	
<3.0	0	0	0	0	0.48	0	0	

Table 4 The percentage of plots with a different absolute residual value of the maturity class, calculated based on NDRE, within a total number of

Table 5 Percentage of plots with a different absolute residual value of the maturity class, calculated based on NDRE using the equation from Fig. 8a for potato A-clones, within a total number of plots with a particular maturity class for potato C-clones

Range of the absolute	Maturity class						
residual value	1	2	3	4	5		
≥0.5	0.00	4.3	71.4	50.0	22.2		
0.51-1.0	0.0	43.5	21.4	50.0	33.3		
1.01-1.50	18.2	26.1	7.14	0.0	44.4		
1.51-2.0	59.1	26.1	0.0	0.0	0.0		
2.01-2.5	22.7	0.0	0.0	0.0	0.0		

plants close to drying (MC of 0.5–1 and 2) and of high green biomass (MC of 6 and 7).

The situation was different for the potato B-clones (Table 4), as a similar and high percentage of plots of MCs from 2 through 6 were assigned MC using NDRE with a low absolute residual value, i.e., below 0.5 and in the range of 0.51–1.0. The plots evaluated by the breeder as MCs 1 and 7 were assigned to the MC using NDRE with the lowest accuracy.

Comparing the results for both potato trials, the MC can be more accurately assigned using NDRE to the 2-row plots of the potato B-clones than to the 1-row plots of the potato A-clones.

Validation of the NDRE Usefulness for Remote Maturity Class Estimation

Equations from Fig. 8a (potato A-clones) and 8c (potato B-clones) were used to estimate the MC of the potato C-clones based on the NDRE values derived from the UAV imagery taken on the same day (July 26, 2023) when the breeder determined the MC class. The MAE values of MC estimation for the 120 validation plots were 1.09 and 0.81 when the equations from Fig. 8a and 8c for potato A and B-clones were used, respectively (data not presented). Maturity classes 3 and 4 using NDRE derived from the potato C-clones and the equation from Fig. 8a developed for potato A-clones were calculated with the lowest MAE. This is because 71.4% and 50% of the plots, with MCs of 3 and 4, respectively, were assigned to the MC with the absolute residual value of ≥ 0.50 (Table 5).

In the case of MC evaluation using NDRE and the equation from Fig. 8c developed for potato B-clones, the lowest MAE was obtained for MCs of 2, 3, and 4 when 52.2%, 50%, and 50% of the plots, respectively, were assigned to the MC with the absolute residual value of ≥ 0.50 (Table 6). The highest error was made in the case of MCs of 1 and 5 evaluated using both questions from Fig. 8a and 8c. It is worth adding that in the case of MC 1 and 5, forty-four

and nine plots belonged to these MCs, respectively. Therefore, we assume that the low accuracy of the above MCs was unrelated to the number of samples in the MCs but was associated with potato canopy greenness. This means that the greatest divergence between the MC evaluated by the breeder and using UAV imagery was observed for plots with low and high chlorophyll content (extreme levels of senescence) in the potato plants that were indirectly evaluated by low and high NDRE values, respectively.

UAV imagery of larger plots of the potato B-clones was characterized by lower MAE of MC estimation using NDRE than potato A-clones (Sect. 3.6). Therefore, the NDRE vs. MC algorithm derived for the former plots gave better results (lower MAE of 0.81) when the NDRE obtained for the potato C-clones was used for validation. The number of plots with MAE higher than 1 was 40 (33.3%). In twentyfour of the 40 plots, the MC of 1 assigned by the breeder was overestimated (a negative value of residuals) using the validation algorithm. In seven of the 40 plots, the MC of 3 and 5 assigned by the breeder was underestimated (a positive value of residuals) using the validation algorithm. This means that the potato plots of the new potato varieties with low plant coverage were often remotely assigned higher MC than by the breeder. In contrast, the underestimation of the MC using remote methods was often related to the potato plots of the new varieties with high plant coverage.

Limitations of Maturity Class Evaluation Using Vegetation Indices

Images taken with a cell phone camera were used to closely examine neighboring potato plots characterized by a significantly different MC prediction error based on the NDRE. Among the three potato plots of the A-clones captured in Fig. 11b, the middle plot with the most uniform canopy was assigned MC with the lowest error of 0.894 compared to the MC of 6 assigned by the breeder. The crop canopy of the plot in Fig. 11a was partially damaged by a tractor during a fungicide spray. That is why the MC of this plot was probably underestimated at 2.871 using NDRE versus MC

 Table 6
 Percentage of plots with a different absolute residual value of the maturity class, calculated based on NDRE using the equation from Fig. 8c for potato B-clones, within a total number of plots with a particular maturity class for potato C-clones

Range of the	Maturity class						
absolute residual	1	2	3	4	5		
≥0.5	6.82	52.2	50.0	50.0	33.3		
0.51-1.0	38.6	17.4	40.5	0.00	22.2		
1.01-1.50	34.1	26.1	9.52	50.0	33.3		
1.51-2.0	13.6	4.3	0.0	0.0	11.1		
2.01-2.5	6.8	0.0	0.0	0.0	0.0		

Fig. 11 Variation in potato crop appearance of the same three neighboring plots of A-clones, visible on UAV image (top) and overhead, cell phone images (bottom), characterized by a different MC prediction error based on NDRE: **a**) high – 2.871; **b**) low – 0.894, and **c**) medium – 1.458. The red arrow indicates which UAV image refers to which phone image of the same plot



6 given by the breeder, who probably rated the plot based on the biomass present. This was because the calculation of the VI value derived from the UAV image is based on the amount of light reflected from the crop and the surrounding soil within the ROI seen within the red-dotted frame. However, the breeder might have evaluated the MC of the plot by paying more attention to the living green parts of the potato crop, not considering the whole crop seen within the frame. In the case of the potato plot shown in Fig. 11c, the MC was overestimated by 1.458 using NDRE versus the MC 3 assigned by the breeder. The UAV image cannot discriminate between vine overlap between plots. In contrast,

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Fig. 12 Variation in potato crop appearance of the same three neighboring plots of B-clones, visible on a UAV image (top) and overhead, cell phone images (bottom), characterized by a different MC prediction error based on NDRE: a) high - 1.576; b) medium - 0.704and c) medium - 0.735. The red arrow indicates which UAV image refers to which phone image of the same plot

the breeder can do this by making notes on the vine habits of the potato clones to improve the accuracy of potato MC estimation remotely.

In general, independently from the VI used, the MC was predicted to have a lower MAE value for the potato B-clones than for the A-clones (Table 2). This was likely the consequence of the more uniform canopy of the two-row versus the one-row plots. In Fig. 12, there are examples of two neighboring B-Clone plots assigned MC 1 by the breeder but remotely evaluated with the use of NDRE as 2.576 (1 plus 1.576) – Fig. 12a and 1.704 (1 plus 0.704) – Fig. 12b. These

evaluations go together with the crop canopy appearance on the cell phone image, as the plants in Fig. 12b look drier than in Fig. 12a, which has also been captured in a UAV image. In Fig. 12c, the potato MC was determined remotely with a lower error of 0.735 because the canopy looks uniform on both the UAV and the cell phone image.

The neighboring plots of the new potato genotypes, shown in Fig. 13, look very different regarding uniformity of plant distribution and shade of greenness. For example, plots number 1284 and 1294 were assigned the same MC of 2 by the breeder, but the NDRE-based algorithm assigned



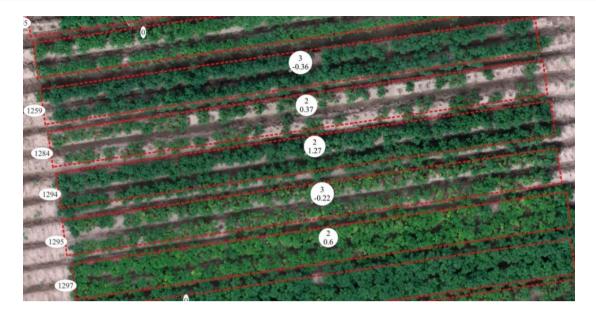


Fig. 13 Variation in potato crop appearance of five neighboring plots of the potato C-clones, visible on a UAV image. The numbers on the left stand for a plot number, the upper number in the middle

represents MC, and the lower number indicates the absolute residual value of MC evaluated using the NDRE and the equation from Fig. 8a developed for potato A-clones

an MC of 2.37 (2 plus 0.37) for plot 1284 and an MC of 3.27 (2 plus 1.27) for plot 1294. This happens so because the coverage of the soil by the potato plants within plot 1284 was much lower than within plot 1294. The lower number of plants within the former plot was caused by removing some diseased plants during the negative selection. The breeder assigned the MC based on the appearance of the healthy potato plants, not considering the reduced number of plants. However, the NDRE-based algorithm took into account both the lowered coverage of the soil by the potato canopy due to the removal of diseased plants and the color of that potato genotype. While vegetation indices like NDRE provide valuable information on the overall status of the plots, it is limited to only the spectral characteristics of the plots. Machine learning can potentially enhance the analysis by integrating a broader range of data to identify subtle variations of features that are indicative of the maturity of the potato genotypes (Psiroukis et al. 2022; Zhang et al. 2019).

Currently, there are no tools to speed up the negative selection (discrimination between virus-infected and healthy plants) done by the breeder and the technical staff's elimination of the infected potato plants. However, using UAV imagery can automate the evaluation of vine maturity in potato breeding trials, which is done about two weeks after the work mentioned above. Moreover, acquiring UAV imagery before the negative selection could be an additional source of information for the breeder to evaluate the performance of the potato genotypes in terms of other potato traits, e.g., resistance to diseases.

Insufficient rainfall during most of the growing season could have accelerated the yellowing of the potato canopy and caused faster maturity of some genotypes. For example, one of the standard, early varieties, *Gala*, matures earlier in unfavorable conditions (personal communication with the breeder). However, this should not limit the applicability of the UAV for MC evaluation; it should ensure that one considers the growing conditions of the potato genotypes and makes some adjustments in the developed procedure of the automated MC evaluation.

Conclusions

This study provided evidence that VIs derived from UAV imagery can be used to remotely assign MCs to potato plants grown in breeding trials. Higher accuracies (lower MAE) with the remote evaluation of the MCs were achieved for the potato B-clones than for the A-clones as well as for the standard potato varieties grown among the A and B-clones. This is probably because it is more challenging to correctly assign MC to the single-row plots of A-clones and standard varieties. Moreover, the genotypes

grown in the potato B-clones trials represent a higher stage of a multi-year breeding program because they are selected from the potato A-clones grown in the previous year. Consequently, the potato B-clones trials cover a lower range of MCs than the potato A-clones and should also be characterized by a narrower range of VI values. It was often challenging to interpret low NDVI (the most commonly used VI) because if the potato canopy is less green, it may be more mature, but at the same time if there is less biomass, it may mean it is a younger plant. With high NDVI values, there is higher saturation and generally less variability because of the similarities between large amounts of biomass.

Among the tested VIs, the NDRE allowed for potato MC evaluation with the lowest MAE. Using NDRE for MC estimation on a validation dataset, resulted in an MC estimate with a 0.81 mean absolute error. Potato vines bending over the neighboring row of potato plants, causing vine overlap between plots, vines damaged by tractor tires during field operations (during spray), and the variability (non-uniformity caused by the removal of diseased plants during the negative selection) of the potato canopy within the region of interest were found to be the main factors limiting an accurate evaluation of the potato MC by both visual (a reference) and remote (UAV image-based) methods. The future line of work will explore machine learning to classify the potato genotypes from UAV-derived images and determine the relationship between manually derived visual potato canopy status estimation and machine learning-derived classification. The UAV-derived imagery could also be used to discriminate the potato genotype differences during early growth regarding other multiple plant traits.

The VIs are not a single measure of a structural and biochemically specific canopy variable (Haboudane et al. 2004). Therefore, the RGB images derived from the potato breeding trials may also be used for machine learningderived classification.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Data Availability Data is available upon request.

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