APPLICATION OF GROUND-BASED HIGH-THROUGHPUT PHENOTYPING PLATFORMS IN CEREAL BREEDING – A REVIEW

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Abstract
Cereals are the most widely grown crops in Europe, accounting for 53.8% of total crop area. Today's farmers are facing serious challenges – climate change, resource scarcity and population growth, which can have a negative impact on the quantity and cost of agricultural production. It is therefore important to focus on the development of cereal breeding programmes and the introduction of new technologies, including High-Throughput phenotyping methods. The aim of this paper was to analyse the recent scientific literature on the current use of ground-based sensors applied in High-Throughput Phenotyping Platforms (HTPPs) for the assessment and analysis of morphological and physiological traits in cereals and for the selection of high-yielding genotypes. This enables the breeder to asses and identify genotypes of interest more quickly and accurately at different stages of plant development and in larger field and laboratory trials than with traditional breeding methods. The paper also provides information on the potential of using ground-based HTPPs, the most important methodological principles in setting up trials and measuring traits to ensure the accuracy of the assessments and the processing and interpretation of the results.
Key words: sensors, morphological traits, physiological traits, yield, correlation.

Introduction

Cereals are among the most widely grown crops in Europe. Compared to other crops, cereals account for 53.8% of the EU's cereal area in 2020 (Eurostat, 2020), with wheat (*Triticum aestivum* L.) accounting for almost 45%, and barley (*Hordeum vulgare* L.*)* 20% of European cereal production (Eurostat, 2020). Wheat (498 800 ha) was also the largest cereal area in Latvia in 2020, followed by oats (*Avena sativa* L.) (98 900 ha) and barley (75 300 ha) (CSB database, 2021).

Agriculture faces several challenges at the same time: climate change, resource scarcity and rising costs, and population growth. Although it has been predicted in the past that global wheat production will increasingly expand to northern regions (Ortiz *et al*., 2008), climate change has tended to reduce cereal productivity and quality in recent years. In recent years, droughts and record-high temperatures have been observed across Europe (NOAA National Centers for Environmental Information, 2020), significantly reducing cereal yields, including the Baltic States (Eurostat, 2020).

Cereal variety breeding, which results in the development of new and high-yielding varieties, is one of the most important resources to meet the demand for cereal inputs to increase food production. Studies confirm the importance of breeding for yield gains, changes in productivity – related traits and disease resistance (Laidig *et al*., 2021). Therefore, in the context of climate change, as well as the political and social imperative to produce more environmentally friendly food commodities, the progress of cereal breeding is also receiving constant attention (Laidig *et al*., 2021). The greatest challenge in crop breeding research in the 21st century is the ability to predict yields that are as close as possible to the genetic potential of a variety. Although genotyping efficiency has improved considerably thanks to new advances in DNA sequencing, methods for assessing plant traits (phenotype) have evolved relatively slowly over the last 30 years, and factors affecting phenotyping efficiency limit the breeder's ability to assess the genetics of quantitative traits, particularly those related to yield and stress tolerance (White *et al*., 2012). Therefore, the introduction and use of innovative phenotype – based selection techniques for breeding of new varieties is a current research area to improve yield and tolerance to abiotic and biotic factors under changing climatic conditions (Rubiales *et al*., 2021).

In breeding programmes using traditional phenotyping methods, breeders rely mainly on the evaluation of the traits of interest using visual and manual phenotyping methods. This is time-consuming, labour-intensive and it requires large human resources to select genotypes of interest from large hybrid populations and breeding nurseries. In contrast, high-resolution phenotyping methods can greatly increase the ability to make observations and quantify traits in field trials and breeding nurseries on a much larger scale (Reynolds, Chapman, & Crespo-Herrera, 2020; Yang *et al*., 2020). Nowadays, plant phenotyping is seen as a new research direction that provides important information on genotype – environment interactions, focused on selecting productive plants suitable for given growing conditions. Moreover, these studies mostly use non-invasive and digital technologies (Costa *et al*., 2019). The use of High-Throughput plant phenotyping platforms (HTPPs) in cereal breeding programmes makes it possible to identify superior genotypes and thus achieve better results in the breeding process (Würschum, 2019). Due to the advantage of such phenotyping technologies, it is possible to replace subjective trait evaluations quickly and efficiently, and their application in plant breeding to select the best genotypes is seen as an important prerequisite for continuous grain yield progress in the future (Deery & Jones, 2021).

HTPP's are facilities on which various sensors and data collection systems are deployed to allow the breeder to assess phenotypes for various traits in large-scale trials (Li *et al*., 2021). The range of available phenotyping platforms is very wide and can be applied in field, greenhouse or laboratory trials using stationary, vehicle-based, self-propelled, portable or aerial platforms. This review focuses on a summary of research on the use of ground-based high-resolution methods for the evaluation of morphological and physiological traits and grain yield, as it is important in breeding to obtain phenotypic data for different genotypes that are as close as possible to real field conditions.

The aim of this paper was to analyse the recent scientific literature on the current use of ground-based sensors applied in High-Throughput Phenotyping Platforms (HTPPs) for the assessment and analysis of morphological and physiological traits in cereals and for the selection of high-yielding genotypes.

Materials and Methods

The present study was carried out using the monographic method to review different precise phenotyping methods provided for identifying morphological and physiological traits. The scientific literature from different journals and monographs has been used from research in Australia, Sweden, Germany, Poland, USA, Spain, Canada, China, Korea and India.

Results and Discussion

Characterization of ground-based HTPP

High-Throughput phenotyping involves specific tools that enable complex assessment of plant morphological and physiological traits at the organ, plant, canopy and even population levels (Li, Quan, & Song, 2021). To assess these different traits, different ground-based HTPPs have been developed, equipped with one or most commonly several combined spectral sensors. The most widely used of which are visible spectrum or RGB (red, green, blue) cameras (Deery *et al*., 2021), light detection and ranging or LIDAR (Deery *et al*., 2021; Lin, 2015), multispectral or hyperspectral cameras and thermal cameras (Bai *et al*., 2016; Kim *et al*., 2021).

Depending on the light source used, sensors are divided into two groups – active and passive sensors. Active sensors can be used in different lighting conditions and this does not affect the accuracy of the data obtained, as they use different independent light sources such as LED lamps as the illumination source (Kim *et al.*, 2016). One of the active sensors frequently used in studies is the GreenSeeker® sensor (Trimble, Sunnyvale, California, USA) which can measure the Normalized Difference Vegetation Index (NDVI) (Deery *et al.*, 2021). Passive sensors (RGB cameras), on the other hand, are dependent on sunlight, which affects their usability (Barmeier & Schmidhalter, 2017).

One of the most widely used phenotyping tools in the breeding programmes of cereal varieties are RGB image-based phenotyping platforms (Zhang & Zhang, 2018), which are typically used as a base sensor in combination with other types of additional sensors (Kim *et al.*, 2021). The main advantages of using RGB cameras compared to spectral sensors are their relative ease of use, low acquisition and maintenance costs (Kim *et al.*, 2021; Prey, von Bloh, & Schmidhalter, 2018). Multispectral and hyperspectral cameras, on the other hand, can provide higher image resolution, but their costs are considerably higher than for RGB cameras (Morgounov *et al*., 2014). Hyperspectral cameras can detect various stress symptoms in plants, including early drought stress symptoms that cannot be assessed visually (Kim *et al.*, 2021).

The literature indicates that each type of sensor has its optimal application time depending on the stage of plant development, as the accuracy of the measurement results can be affected by light reflection from the soil. For cereals, RGB is recommended for traits' assessment at early stages of plant development, while spectral measurements are recommended for cameras at later stages (Prey, von Bloh & Schmidhalter, 2018). Sensor performance can also be affected by various environmental changes such as sunlight, temperature, humidity and wind. To ensure the accuracy of the data obtained, it is recommended that measurements with RGB cameras are taken at a certain height above the canopy vegetation between 10 am and 2 pm, as the low sunlight angle in the afternoon causes shadows to form on the canopy (Bai *et al.*, 2016; Fernandez-Gallego *et al*., 2018). For better interpretation of the data when measurements are made under changing environmental conditions, it is recommended to also collect data on solar radiation, air temperature and relative humidity (Bai *et al*., 2016).

The use of HTPPs requires the development of state-of-the-art information technologies, as phenotyping of plants with different sensors generates large amounts of data, and the processing of the resulting data is complex (Rosenqvist *et al*., 2019), requires higher-capacity computers for image pre-processing and interpretation of the results (Bai *et al*., 2016). Precise data structure, definition of algorithms and experiments, and data sharing are crucial as well (Rosenqvist *et al*., 2019).

Morphological traits assessment

Studies on plant greenness traits are of current interest as aboveground biomass, surface transpiration, photosynthetic potential and light interception are closely related to all green parts of plant organs such as leaves, ears and stems (Pask *et al*., 2012). **Early vigour (EV)** is a physiological trait that is the ability of plants to rapidly build leaf area and aboveground biomass during the early stages of development, thereby reducing water evaporation from the soil (Mullan & Reynolds, 2010) and increasing water use efficiency and competitiveness with weeds (Pask *et al*., 2012). EV can be used in cereal breeding programmes as a selection criterion to estimate plant biomass and predict plant productivity (Kipp *et al*., 2013). In traditional methods, EV is assessed visually (in scores), which is strongly influenced by subjective evaluation. If a larger number of genotypes needs to be assessed quickly and accurately, RGB cameras can be used from HTPPs, and these images are analysed in dedicated computer programs (Khadka *et al*., 2020). Spectral camera measurements are recommended especially in large field trials; the calculated Spectral Plant Vigour Index (EPVI) showed a consistent positive correlation with EV at the plant emergence stage over the years (Kipp *et al*., 2013).

Canopy ground cover (CGC) is the proportion of soil covered by the plant canopy, a trait influenced by both the morphology of the plant canopy and the rapid growth capacity of the plant up to and during the tillering stage. In cereal crops, CGC reduces soil water evaporation, soil erosion and nitrogen leaching into groundwater (Prabhakaraa, Hively and McCartyc, 2015) and in wheat also increases competitiveness with weeds (Feledyn-Szewczyk, Jończyk, & Berbeć, 2013). Trait CGC in cereals at both early and late stages of plant development is assessed using visual and HTPP assessment methods (Deery *et al*., 2014; Jimenez-Berni *et al*., 2018) with a strong correlation between the two methods found in spring wheat (Walter *et al*., 2019). In a study by Deery *et al*. (2014), CGC and canopy colour with an RGB camera were evaluated, calculating a greenness index. Another study used a spectral camera to assess this trait in wheat, finding a close correlation (r=0.93) between NDVI and CGC (Prabhakaraa, Hively, & McCartyc, 2015). A study by Deery *et al*. (2021) measured NDVI with GreenSeeker® during the emergence and tillering phases of plants, showing the least variation between measurements, unaffected by differences in light conditions and the presence of dew. When measuring CGC, it should be taken into account that the result may be influenced by intensity of light reflection from canopy, which depends on the structural and optical characteristics of the plants, as well as on the structural parameters of canopy such as row spacing, plant density and seeding direction (Kuester & Spengler, 2018).

Aboveground biomass (AGB) is a trait that represents vegetative plant mass per unit area and can be used to predict grain yield in cereals, including under drought stress (Morgounov *et al*., 2014). The detection of this trait by traditional methods is destructive, time and resource consuming, limiting the evaluation of large number of genotypes. Literature suggests that in barley the NDVI correlates well with AGB during intensive plant growth (Calera, Gonzalez-Piqueras, & Melia, 2004). According to the available information in the literature, significant differences in the change of NDVI index from the tillering to the flowering phase in wheat were found for high and low yielding genotypes (Morgounov *et al*., 2014). Bai *et al*. (2016) used RGB images to detect AGB at early plant stage (before plant tillering) by determining the proportion of green pixel fraction (GPF).

Plant height (PH) is closely related to AGB, which can be influenced by air temperature and soil moisture regime, especially until flowering. Kronenberg *et al*. (2020) points out that at the tillering stage, under conditions of elevated temperature and moisture stress, the process of stem elongation may differ between different wheat genotypes with consequent effects on both AGB and plant productivity. In different studies the height of the cereal canopy was assessed using a soil laser scanner (Kronenberg *et al*., 2020), an ultrasonic sensor (Bai *et al*., 2016), and LIDAR (Jimenez-Berni *et al*., 2018).

The leaf area index (LAI) is a trait that shows the ratio of green leaf area per unit of land surface, and it strongly correlates with cereal AGB and grain yield (Hasan, Sawut, & Chen, 2019). The LAI can estimate how much of a plant's leaf surface area captures solar radiation (González-Sanpedro *et al*., 2008). More accurate LAI measurements can be obtained with optical hand-held instruments, but it is labour and time consuming (González-Sanpedro *et al*., 2008; Nie *et al*., 2016). Ground-based HTPPs such as RGB cameras (Kim *et al*., 2021) and LIDAR or spectral sensors for NDVI detection can be used (Deery *et al*., 2014).

Physiological traits assesment

Physiological-functional traits include various measures of plant functions, such as photosynthesis, respiration, stress tolerance and plant water relations (respiratory function and transpiration) (Zhang & Zhang, 2018), canopy temperature (Li *et al*., 2019) and leaf senescence (Fernandez-Gallego *et al*., 2019).

Chlorophyll fluorescence is a characteristic of **photosynthetic rate (PR)** and indicates the response of plants under drought stress, usually detected by using fluorescence sensors. This method can measure photosynthetic rate, conductance and gas exchange in leaves of plants under drought stress (Kim *et al*., 2021). High-Throughput phenotyping techniques use change-coupled cameras (CCD) to assess changes in various physiological parameters. It has been noted in literature that using fluorescence analysis makes these studies cumbersome to conduct in the field, as preparatory steps such as adjusting darkness and ensuring constant lighting conditions are required (Tietz *et al*., 2017). In the field, HTPPs can determine the photosynthetic area of the canopy by calculating vegetative indices such as relative green area (GA) and relative greener green area (GGA) (Fernandez-Gallego *et al*., 2019).

Canopy temperature (CT) for cereal describes the water status of the plant and is also used in breeding programmes to assess the response of genotypes under drought stress (Mason & Singh, 2014). CT is influenced by the genotype of the variety, which is determined by factors such as shoot morphology, root depth and root biomass, and external environmental factors such as solar radiation, soil moisture, wind speed, temperature and relative humidity (Li *et al*., 2019; Reynolds, Pask, & Mullan, 2012). CT is traditionally determined with an infrared thermometer. However, the result of this method in large-scale field trials can be affected by weather fluctuations, and the method is labour and time consuming (Deery *et al*., 2016). With HTPPs measurements of CT for cereal crops is recommended to be measured with a thermal camera (between 12 am until 2 pm), a thermal infrared radiometer (Bai *et al*., 2016) or by calculating the NDVI of the canopy at the beginning of the grain filling period (Li *et al*., 2019).

Leaf senescence (LS) is a trait characterised by the yellowing of the green leaves of plants. In cereals, it can be used to assess the effect of biotic (diseases) and abiotic stress (temperature, moisture, nutrient supply) on plants (Distelfeld, Avni, & Fischer, 2014). When LS occurs prematurely in plants, it causes yield and grain quality losses (Gregersen *et al*., 2013). Therefore, in cereal breeding programmes, the trait 'stay-green' (de Souza Luche *et al*., 2015; Lopes & Reynolds, 2012) is used to compare varieties for LS, which is usually assessed visually in scores. In HTPPs measurements of LS for cereals are assessed using spectral sensors by calculating NDVI, which simultaneously provides information in the degree of greenness level of the canopy (Fernandez-Gallego *et al*., 2018) and RGB cameras by calculating vegetative indices – plant senescence reflectance index (Fernandez-Gallego *et al*., 2019).

Nitrogen use efficiency (NUE) is a trait that is receiving increasing attention in the breeding programmes of cereal varieties (Nehe *et al*., 2020; Nguyen *et al*., 2019). A positive aspect of nitrogen use efficient varieties is their ability to maintain grain yield and quality even at reduced nitrogen fertilizer rates. Accurate assessment of this trait is resource-intensive, so the use of HTPPs is an important alternative for comparing NUE between varieties. In N-use related studies, HTPPs digital imaging aims at automated measurements of plant growth, organ development, physiological parameters and biochemical components (Nguyen & Kant, 2018). The application of HTPP methods for the identification of NUE is still under investigation, at first adopting them under controlled cultivation conditions. Banerjee *et al*. (2020) used RGB and hyperspectral imaging methods to identify NUE, 47 vegetative indices described in the literature were tested of which the Transformed Chlorophyll Absorption Reflectance Index (TCARI), the Vogelmann Red Edge Indices (VOG), Miller Index (ZMI) showed the highest correlation with chlorophyll levels. In this study, an improved vegetative index, the normalized difference chlorophyll index in wheat ($NDCI_W$) was developed, which together with digital plant biomass measurements are recommended for use as biomarkers for the selection of N-responsive wheat genotypes during the vegetative stages of plant development.

Grain yield and its components

As grain yield is a complex trait, the use of ground-based HTPP for estimating yield components is also recommended in grain breeding studies (Fernandez-Gallego *et al*., 2018; Hasan *et al*., 2018). **Spike number (SN)** or spike density per unit area is a trait that is formed by the interaction between yield components such as the number of plants and their productive tillering. The rapid assessment of this trait in large-scale studies helps the breeder to predict grain yield early, and it is recommended as a selection criterion in breeding programmes to select high-yielding genotypes (Fernandez-Gallego *et al*., 2018). Fernandez-Gallego *et al*. (2018) used an RGB camera to count spikes in durum wheat (*Triticum turgidum* L. subsp. *durum*), taking one image for each plot at a height of 1 m above the canopy. Automatic image processing was then carried out with algorithms developed for different wheat varieties and plant development stages. In this study, the SN per unit area assessed at flowering correlated better with grain yield than when this trait was assessed at later stages of plant development. The result may be influenced by the senescence of canopy, which complicates the image processing. Also, Hasan *et al*. (2018) recommends using an RGB camera, but only in an oblique position relative to the field surface. It was concluded that in this way, images could be better analysed for various characteristics of spikes such as texture, colour and shape, productivity and disease resistance.

Grain yield (GY) is a performance trait that is commonly the focus in the cereal breeding programmes (Bai *et al*., 2016). Grain yield prediction is a complex task, so it is essential for the breeder to find an efficient method for its accurate and early estimation. Studies using HTPPs with different sensors measure grain yield in cereal crops and analyse whether there are positive correlations between vegetative indices and grain yield, with the aim of finding out which of them could be used for GY prediction. For grain yield estimation Normalised Difference Vegetative Index (NDVI) (Bai *et al*., 2016; Christopher *et al*., 2014; Morgounov *et al*., 2014; Naser *et al*., 2020; Sultana *et al*., 2014), Green Area (GA), Greener Green Area (GGA), Normalized Green-Red Difference Index (NGRDI), and Triangular Greenness Index (TGI) (Fernandez-Gallego *et al*., 2019) are more widely recommended vegetative indices.

Conclusions

Sensors used in ground-based High-Throughput phenotyping platforms (HTPPs), including visible spectral (RGB) and hyperspectral cameras, can be used to assess and analyse morphological and physiological traits and select high-yielding genotypes in cereal breeding programmes. HTPPs methods enable the breeder to evaluate and identify genotypes of interest more quickly and accurately at different stages of plant development, including early stages, and in larger field and laboratory trials than traditional breeding methods. When working with HTPPs methods, depending on the type of sensor used, it is important to follow certain methodological principles for setting up the experiments, taking the measurements, processing the data and interpreting the results. The HTPPs studies carried out so far have mainly focused on wheat, so it is relevant to test the application of these methods also to other cereal species. In cereal breeding programmes, especially under field conditions, further research is needed to identify accurate biomarkers for the identification of nitrogen-use efficient and high-yielding genotypes by HTPPs methods.

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