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Application of RGB-Imaging techniques for high-throughput plant phenotyping- A Review

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ABSTRACT

High-throughput plant phenotyping plays an important role in plant breeding, identifying superior genotypes in a fast and accurate manner. Consequently, researchers are seeking new image-based plant phenotyping strategies to enhance phenotyping efficiency. RGB (red, green, blue) sensors and their applications offer a cost-effective and accessible approach while maintaining the advantageous characteristics of high-throughput phenotyping technologies. Various RGB image-based indices are now available to measure diverse phenotypic traits accurately. Despite a wide range of advantages, some limitations reduce the accuracy of the method. We systematically reviewed scientific articles published between 2000 and 2024 to ascertain the significance, available knowledge, and gaps in the domain of RGB image-based plant phenotyping. This review paper provides a comprehensive survey on the significance of current RGB imaging technologies and their applications in plant phenotyping, emphasizing their advantages and limitations. RGB image-based plant phenotyping demonstrates considerable accuracy in the estimation of morphological traits. However, this technique gives significantly lower accuracy for physiological traits compared with other sensors. Furthermore, variation of light conditions, varied backgrounds, and overlapping are major drawbacks of this technique. Future studies should focus on the development of precise image acquisition systems, advanced image processing techniques (including image segmentation), the identification of novel color parameters, the implementation of robust artificial intelligence and machine learning models, and the integration of complementary sensor technologies to address existing challenges.

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Introduction

Crop improvement programs play a crucial role in modern agriculture by enhancing crop productivity to meet the rising global food demand driven by continuous population growth. In 2023, worldwide primary crop output reached 9.9 billion tons, exhibiting a 3% increase from 2022 and a 28 percent increase since 2010 (FAO, 2024). However, maintaining food security remains a significant challenge due to various factors that adversely affect agricultural productivity. Consequently, agricultural researchers and plant breeders are increasingly concentrating on utilizing genomics and innovative technologies to improve current plant breeding programs (Walter et al., 2019; Sie et al., 2022), aiming to cultivate plants with increased biomass, enhanced nutritional value, and superior resilience to biotic and abiotic stress (Omari et al., 2020). The advancement of high-throughput phenotyping methods and gene sequencing technology has facilitated the investigation of the genetic frameworks of plant characteristics. Techniques, such as quantitative trait locus (QTL) mapping, candidate-gene association studies, and genome-wide association studies (GWAS), have been employed to investigate various aspects of plant structure, development, and environmental responses, thereby linking phenotypes and genotypes (Mir et al., 2019). The substantial expansion of genetic data in plant biology has required the development of high-throughput phenotyping procedures to address the widening genotype-phenotype gap (Walter et al., 2015). Linking genotype to phenotype enables quicker and more efficient selection of high-yielding, stress-tolerant plants compared to existing traditional methods. (Li et al., 2014). The plant phenotype encompasses all morphological, physiological, and biochemical attributes that reflect a plant's structure, composition, and growth, including agronomic traits such as structure, size, and color, as well as physiological conditions during growth and development (Das

Choudhury et al., 2019). Conventional phenotyping techniques predominantly depend on manual measurements, which are arduous and time-consuming, hindering the collection of extensive phenotypic data from individuals within massive populations. Furthermore, manual measurements are inherently subjective and susceptible to errors, compromising the accuracy and reliability of the data (Montes et al., 2007; Xiao et al., 2021). Imaging techniques such as visible light imaging, fluorescence imaging, thermal imaging, near-infrared imaging, hyperspectral imaging, three-dimensional (3D) imaging, laser imaging, magnetic resonance imaging (MRI), positron emission tomography (PET), and computed tomography (CT) are extensively utilized in plant phenotyping. The identification of limitations and existing gaps in the knowledge is crucial to expand the accuracy and efficiency in image-based plant phenotyping techniques. This review provides an overview of RGB imaging techniques in plant phenotyping.

Methodology

We conducted a systematic review (Figure 1) of the scientific articles published between 2000 and 2024 to examine the development, application, and limitations of RGB image-based plant phenotyping techniques. The literature search was performed using three major databases: Scopus, Web of Science, and Google Scholar. Keywords such as "RGB imaging", "plant phenotyping", "high-throughput phenotyping", "morphological trait detection", and "remote sensing in agriculture" were used to identify relevant peer-reviewed journal articles, review papers, and conference proceedings. This review synthesizes the current advancements and challenges associated with RGB imaging in plant phenotyping and to highlight its potential. Additionally, the review aims to identify knowledge gaps and propose future research directions that can enhance the utility of RGB imaging for trait assessment in diverse plant species and environmental conditions.

Plant Phenotyping

Plant phenotyping is the thorough evaluation of complex plant characteristics, including growth, development, tolerance, resistance, architecture, physiology, ecology, and yield, as well as the fundamental measurement of specific quantitative factors that underlie these complex traits. Furthermore, it is an essential tool for analyzing and understanding plant-environment interactions, which can be applied in crop management strategies (Houle et al., 2010; Walter et al., 2017). Phenotyping categorizes crop features into three classifications: morphological, physiological, and pathological. Morphological traits are direct indicators of the shapes and structures of plant organs, including characteristics such as shape, color, texture, pattern, and size. Physiological traits reflect the functional processes of plant organs, including photosynthesis, respiration, and nutrient uptake, hormonal activity, stress tolerance, and water relations (Singh et al., 2024). Image-based phenotyping, mainly hyperspectral, thermal, and RGB imaging, has shown potential in detecting plant diseases early by tracking small variations in spectral or thermal variables between healthy and infected tissues (Mahlein et al., 2012). High-throughput phenotyping may be conducted on a specific collection of traits. In this context, "throughput" in plant phenotyping denotes the quantity of individual units assessed at various structural levels inside plants. Integrating automated, high-throughput phenotyping systems with artificial intelligence has significantly addressed challenges associated with current advanced crop stress phenotyping methods. The concept of phenomics has transformed all procedures in agricultural research and development. It is defined as characterizing the complete array of phenotypes within a given species (Mansoor et al., 2024). In genomics, a sequenced genome is fully defined; conversely, the entire phenome cannot be completely characterized due to its highly dynamic and multidimensional nature. High-throughput phenotyping also offers significant potential for non-destructive and efficient field-based plant phenotyping (Gill et al., 2022).

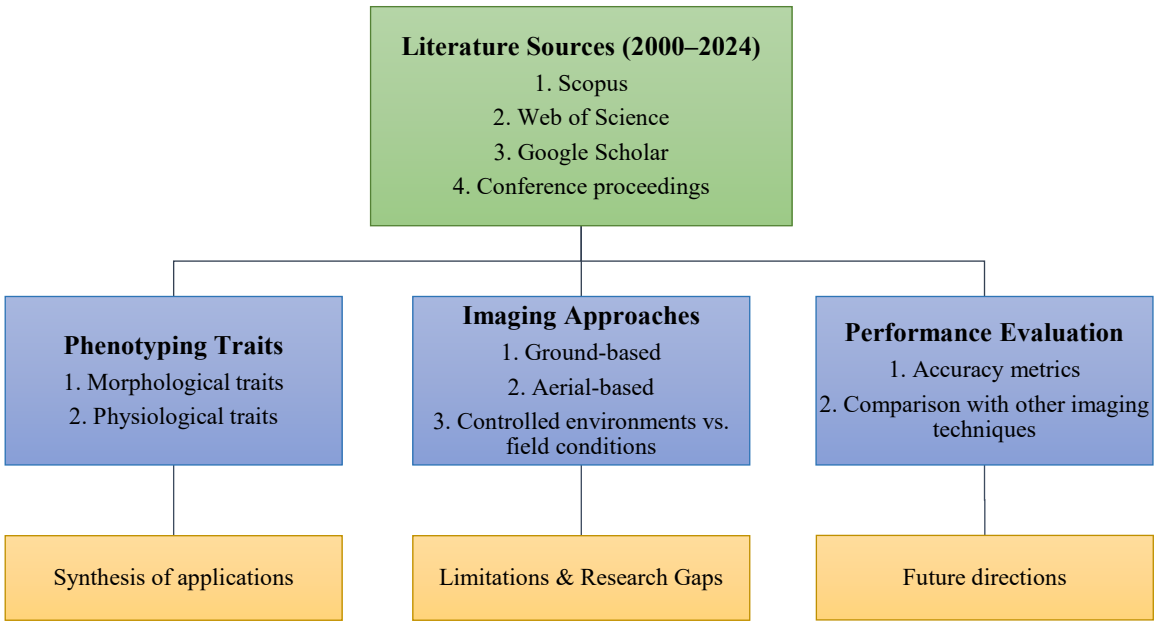

Figure 1. Conceptual framework illustrating the systematic review process

Importance of Imaging Techniques in Plant Phenotyping

Plant phenotyping methods have been undergoing significant transformation since advanced imaging technologies were introduced. These techniques have dramatically improved the ability to observe, quantify, and analyze plant phenotypical traits. Over recent decades, rapid advancements in economically viable sensors and imaging technologies have revolutionized plant phenomics. Furthermore, these advancements have fostered synergy among sensors, imaging methodologies, and analytical tools (Kim & Chung, 2021). This integration has resulted in the development of compact imaging devices suitable for high-throughput phenotyping research (Granier et al., 2006). The complexity of plant phenome analysis remains considerable, owing to the extensive interaction among environmental factors, agricultural practices, and plant genomes. Thus, research, innovation, and communication are essential components for addressing the challenges in plant phenotyping (Fiorani & Schurr, 2013). The key elements of digital phenotyping for objective measurements include real-time data storage with high spatial and temporal resolution, followed by advanced data processing (Kior et al., 2024). Technological advancements can be applied similarly to industrial applications in laboratory or greenhouse settings. The advent of digital photography has significantly influenced the rapid expansion of applications, particularly in machine vision technologies within agriculture (Chen et al., 2002). Consequently, the first commercially accessible digital image processing systems emerged for monitoring specific environmental conditions, including greenhouses, indoor production lines, and encapsulated devices on agricultural machinery, such as the inspection of cereal grains (Davies, 2012). In outdoor environments, driver assistance systems utilizing laser scanning or digital cameras have also been developed; however, accurately detecting crop characteristics under such conditions remains a significant challenge.

Visible Light (RGB) Imaging Techniques in Plant Phenotyping

Contemporary developments in electronics, computer science, and sensor technologies have facilitated the evolution of phenotyping, leading to the introduction of innovative methodologies for measuring phenotypic traits. RGB imaging is widely employed in agricultural research within plant science to assess morphometric and physiological characteristics of plants due to its simplicity and cost-effectiveness (Kolhar & Jagtap, 2021). This imaging approach is typically executed using conventional color cameras that operate within wavelengths of 400 to 750 nm (red, green, and blue) in the electromagnetic spectrum (Figure 2) (Rezzouk et al. 2020; Qiu et al., 2018). Color variations can indicate different phases of plant growth, maturity, senescence, nutrient deficiencies, and the influence of stresses (Kior et al., 2024). In controlled environments such as glasshouses, greenhouses, and screen houses, visible imaging techniques are effective for evaluating leaf biomass, crop characteristics, panicle attributes, growth inhibition and growth rates, leaf physiology and structure, seedling vigor, biomass, kernel morphology, and root structural traits (Li et al., 2014). Furthermore, RGB imaging systems are efficient, non-invasive, and cost-effective tools for evaluating food product quality. They are extensively utilized in food quality control processes, particularly for identifying surface imperfections and performing grading (Liming and Yanchao, 2010). RGB and multispectral cameras are passive sensors that capture images of plants by detecting the spectrum of light reflected from them (Kolhar & Jagtap, 2021). Consequently, any disruptions in solar radiation influence the amount of light reflected by plants, which may subsequently impact imaging quality when these sensors are used in the field, particularly under overcast weather conditions (van et al., 2020). Accurate object identification in images using RGB data enables the separation of target plants from the background. Furthermore, diverse and reliable vegetation indices (Table 1) can be achieved by integrating RGB images with additional sensors that emit specific wavelengths, including near-infrared (Kim & Chung, 2021).

RGB Sensors and Their Application

In contrast to a controlled laboratory setting, measuring plant phenotypes under typical growth conditions remains a significant challenge in field and greenhouse phenotyping. Moreover, findings from controlled environments often differ significantly from the actual field conditions, making it challenging to extrapolate (Ma et al., 2022). Sensors are extensively utilized in phenotyping to measure traits such as moisture content, pigment concentration, photosynthetic rates, plant growth, yield, and adaptation to various abiotic and biotic stresses by analyzing light reflection (Deery et al. 2014). RGB sensors, platforms, and data processing are essential for high-throughput phenotyping procedures.

Optical sensors detect specific wavelength bands of light and convert them into electrical signals, as demonstrated by Holland et al. (2012).

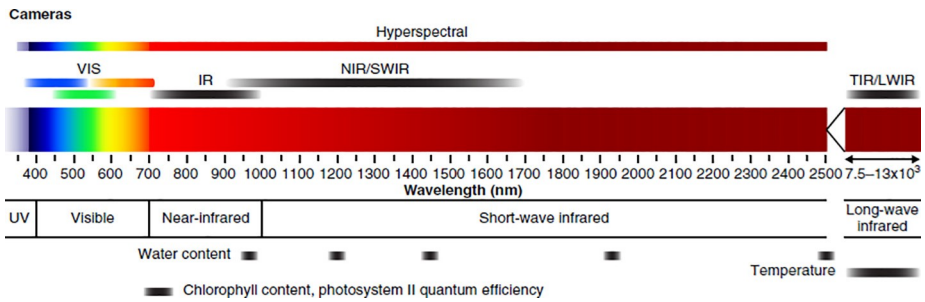

Figure 2. Images capturing sensors and their wave length (Fahlgren et al., 2015)

Table 1. Growth indices that necessitate the RGB spectrum for their determination

Index	Wavelength	Formula	Plant Characteristic	References
Normalized red Coordinate (r)	RGB	$r = \frac{R}{R+G+B}$	Plant height	Feng et al. (2022)
Normalized green coordinate (g)	RGB	$g = \frac{G}{R+G+B}$	SPAD, Shoot nitrogen concentration	Jia et al. (2004)
Normalized blue coordinate (b)	RGB	$b = \frac{B}{R+G+B}$	Leaf area index	Feng et al. (2022)
Normalized difference index (NDI)	RG	$NDI = \frac{r-g}{r+g+0.01}$	Chlorophyll a & b	Widjaja Putra & Soni (2017)
Soil adjusted vegetation index green (RGB) (SAVIgreen)	RG	$SAVIgreen = \frac{(1+L)(g-r)}{(g+r)+L}$ $L = 0.5$	Chlorophyll a & b	
Normalized excess green index (NExG)	RGB	$NExG = 2g - r - b$	Vegetation cover	Rasmussen et al. (2016)
Excess green index (ExG)	RGB	$ExG = 2G - R - B$	SPAD	Liu et al. (2021)
Excess red index (ExR)	RG	$ExR = 1.4g - r$	Aboveground biomass	Feng et al. (2022)
Green leaf index (GLI)	RGB	$GLI = \frac{2G-R-B}{2G+R+B}$	Plant nitrogen concentration	Liu et al. (2023)
Vegetative index (VEG)	RGB	$VEG = \frac{G}{R^a B^{1-a}}$ $a = 0.667$	Aboveground biomass	Cen et al. (2019)

Phenotypic traits are measured using sensors affixed to the platform, providing accurate and uniform quality, after which the phenotypic data are automatically processed according to the user's specified protocol (Cabrera-Bosquet et al., 2012). The technological specifications of visible spectrum image sensors, including light sensitivity, image resolution, and focusing capability, have markedly improved over time (Mahlein, 2016). Moreover, integrations with additional optical sensors provide more precise measurements of vegetation indicators, such as the normalized difference vegetation index (NDVI), and enable the correct separation of plant segments from their backgrounds (Kim et al., 2018). The major advantages of RGB sensors include their affordability, efficiency, and the ability to acquire accurate data cost-effectively. Their feasibility, accessibility, ease of use, and competitive pricing relative to other sensors facilitate applications in precise and rapid morphological phenotyping, in contrast to using more expensive sensor technologies (Araus et al., 2018; Rezzouk et al., 2020). Additionally, the limited range of reflected values for the red, blue, and green bands (380 to 760 nm) compared to other sensors allows for the optimization of relatively few calibrations for band selection and correction (Kim et al. 2018).

Phenotyping with RGB Images

Digital color imaging monitors numerous metrics associated with plant growth rates and offers insights into plant morphology (Table 2). It facilitates the non-invasive assessment of observable plant traits, including shoot biomass, drought stress, salt stress, cold stress, nutrient deficiency, leaf area, stem length, and tiller count (Kolhar & Jagtap, 2021). Hobart et al. (2020) and Guo et al. (2021) reported that applying unmanned aerial vehicle (UAV) platforms equipped with RGB imaging successfully estimated traits such as tree height in apple orchards and plant growth indices in pear orchards, respectively. Several studies have revealed that fixed RGB setups can provide accurate measurements of biomass, leaf area, and morphological indices (Johansen et al., 2020; Jiang & Yang, 2022; Gang et al., 2022). Similarly, Alaguero-Cordovilla et al. (2018) and Brainard et al. (2021) showed that RGB imaging using flatbed scanners or DSLR cameras enabled precise characterization of root system architecture in tomatoes and root shapes in carrots. Furthermore, smartphone-based RGB imaging techniques have been adapted to determine the greenness value of tea leaves and thereby estimate their SPAD indices using broadly accessible devices (Tharuka & Wijerathne, 2024). RGB image-based phenotyping techniques provide substantially accurate results for specific traits compare with other image-based techniques (Table 3). However, while it is ideal for measuring external traits, it gives less accurate physiological traits. Nevertheless, Yadav et al. (2009) have documented that accuracy to some extent. Even in field conditions, UAV-based RGB imaging also has significant results (Luana et al., 2020). Giving robust spectral information, hyperspectral imaging techniques have shown effective detection of physiological traits. Recent studies have resulted in higher accuracy for a wide range of plant physiological traits (Pandey et al., 2017; Ge et al., 2016). However, some studies have exhibited slightly lower R^2 (0.872) for some traits such as relative water content in maize (Rehman et al., 2020). Similarly, the MRI and X-ray imaging techniques have higher accuracy but higher cost for sensors.

Table 2. Comparative overview of RGB imaging-based techniques in plant phenotyping studies

PP	Sensor	GC	Plant Species	Platforms	Phenological Stage	References
Tree height estimation	Consumer RGB camera (24.7 MP APS-C sensor)	F	Apple (*Malus domestica*)	Octocopter UAV with oblique-view camera setup	Post-flowering	Hobart et al. (2020)
Plant growth	RGB camera (DJI Phantom 4 Pro V2.0, 20 MP, 8.8 mm lens)	F	Pear (*Pyrus* spp.)	DJI UAV at 75 m Above Ground Level (AGL)	Mid to late growth	Guo et al. (2021)
Tuber growth	RGB cameras	C	Potato (*Solanum tuberosum*)	High-throughput phenotyping platform (RGB)	From shoot emergence to tuber harvest	Musse et al. (2021)
Biomass (fresh shoot mass)	RGB camera (Zenmuse X3, Sony EXMOR CMOS)	F	Tomato (*Solanum pimpinellifolium*)	DJI Matrice 100 UAV at 13 m AGL	Mid to late season	Johansen et al. (2020)
Fresh weight, dry weight	Intel RealSense D415 stereo RGB-D camera	C	Lettuce (*Lactuca sativa*)	Fixed stereo camera above plant canopy (0.9 m)	Growth to harvestable (7 weeks)	Gang et al. (2022)
Shoot dry weight	RGB cameras (Basler Ace, 5MP)	C	Switchgrass (*Panicum virgatum* L.)	Ag Alumni Seed Phenotyping Facility	From early vegetative to flowering stage	Jiang & Yang (2022)
Root system architecture	Flatbed RGB scanner, Digital camera (Sony DSC-H3, Canon EOS1100D)	C	Tomato (*Solanum lycopersicum*)	Petri dishes with imaging setup	Seedling stage	Alaguero-Cordovilla et al. (2018)
Root shape attributes	DSLR RGB camera (24 mm lens)	F	Carrot (*Daucus carota*)	Custom imaging station with fluorescent lights	Mature root at harvest (107 Days)	Brainard et al. (2021)
Root shape and size	RGB digital camera	F	Cassava (*Manihot esculenta Crantz*)	Field board setup with LED-light	Mature storage root stage	Yonis et al. (2020)
Total carotenoid content	RGB camera (8 MP tablet camera)	F	Cassava (*Manihot esculenta* Crantz)	Custom imaging box with blue background and LED lighting	Mature harvested roots	Rocha et al. (2022)
Leaf greenness	Smartphone camera (resolution of 3120 × 4160 pixels)	F	Tea (*Camellia sinensis* L.)	Custom imaging box with blue background and LED lighting	Harvesting stage	Tharuka & Wijerathne (2024)
Chlorophyll content	RGB camera (13 MP digital camera)	F	Pepper (*Capsicum annuum*)	Field imaging at ~55 cm above canopy	Vegetative to early reproductive stage	Fawzy et al. (2022)
Leaf area	RGB camera (Teli FireWire CSFS20CC2, 1280 px)	C	Sweet pepper (*Capsicum annuum* L.)	Custom trolley-based imaging setup with flash lighting	Vegetative to reproductive growth (up to Week 12)	Horgan et al. (2015)
Leaf inclination angle	Dual RGB cameras + inclinometer	C	String bean	Auto-LIA system with 2 stationary RGB cameras	15 day seedlings	Jiang et al. (2024)
Leaf area and	Conventional RGB camera	F	Grapevine (*Vitis vinifera* L.)	RGB camera at 2m distance	Harvest stage	Diago et al. (2012)
Fruit shape	Digital RGB camera	F	Strawberry (*Fragaria ananassa*)	Custom imaging box with lighting setup	Mature harvested fruit	Feldmann et al. (2020)
Fruit morphology	RGB camera (Canon EOS 20D, 50 mm fixed-focus lens)	F	Tomato (*Solanum lycopersicum* L.)	Imaging box with diffuse light (6000K)	Mature harvested fruit	Zhu et al. (2022)
Shape traits	RGB camera (Manta G-507, 5 MP)	F	Bush bean (*Phaseolus vulgaris* L.)	Closed RGB imaging box with top-down LED	Mature pods and early-to-late seed formation stages	Jollet et al. (2023)
Freezing injury (color index)	Industrial RGB camera (MV-CA050-20GC)	F	Wheat (*Triticum aestivum*)	Near-ground mobile phenotype cabin	Seedling pulling stage	Fu et al. (2021)
Water and Potassium stress	RGB camera (Canon EOS 760D, 24.2 MP)	C	Cassava (*Manihot esculenta* Crantz)	Fixed camera stand in greenhouse	Early vegetative phase	Wasonga et al. (2021)

PP: Phenotype parameters GC: Growing condition; F: Field; C: Control

Table 3. Different image techniques-based plant phenotyping studies

Method	Plant	Method	Accuracy	Imaging condition	Reference
RGB imaging	Potato	Chlorophyll content	RMSE = 8.8, R^2 Green = 0.77, R^2 Red = 0.74, R^2 Blue = 0.42	Control	Yadav et al. (2009)
RGB imaging (UAV RGB Images)	Coffee	Leaf area index	RMSE = 0.475, R^2 = 0.88	Field	Luana et al. (2020)
RGB imaging	Rice	Rice panicle phenotyping	RMSE = 4.94, R^2 = 0.99	Control	Yu et al. (2020)
Hyperspectral imaging	Maize	Leaf water content	RMSE = 3, R^2 = 0.87	Control	Ge et al. (2016)
Hyperspectral imaging	Soybean	Water content Nitrogen content Phosphorous content Potassium content	R^2 = 0.93 R^2 = 0.92 R^2 = 0.83 R^2 = 0.83	Control	Pandey et al. (2017)
Hyperspectral imaging	Maize	Relative water content	RMSE = 10.33, R^2 = 0.872	Control	Rehman et al. (2020)
Infrared imaging	Tobacco	Leaf growth	Accuracy 96%	Control	Dellen et al. (2015)
3D imaging	Strawberry	Fruit height Fruit width Fruit volume	RMSE = 2.83, R^2 = 0.91 RMSE = 1.49, R^2 = 0.94 RMSE = 2.69, R^2 = 0.97	Control	He et al. (2017)
MRI	Maize and Barley	Root fresh weight Root length	R^2 = 0.97 for maize and R^2 = 0.64 for barley R^2 = 0.63 for maize and R^2 = 0.79 for barley	Control	Van Dusschoten et al. (2016)
X-Ray	Rice	Grain weight Grain volume Grain number Grain surface area	R^2= 0.986 R^2 = 0.980 R^2 = 0.944 R^2 = 0.974	Control	Hu et al. (2020)

High-resolution methods such as MRI and X-ray computed tomography (CT) are mostly employed for root phenotyping. MRI applications in plant phenotyping range from seed imaging to fluid imaging. MRI is utilized for quantifying characteristics such as root length, diameter, growth angles, and mass (Van Dusschoten et al., 2016).

Advantages and Limitations of RGB Image-Based Phenotyping

RGB imaging sensors can be employed under a wide range of environmental conditions, including protected and open-field environments, due to its cost-effectiveness and easy accessibility compared to other imaging techniques. Its ability to measure key plant structural and functional indices, such as leaf area, plant height, canopy cover, fruit morphology, and biomass through simple, non-destructive measurements is an imperative advantage (Brainard et al., 2021; Diago et al., 2012; Wasonga et al., 2021). Furthermore, the integration of advanced techniques, such as computer vision and machine learning, along with 3D reconstruction from multi-camera systems, has expanded the accuracy, reduced the time consumption, and enhances the potential of RGB image-based phenotyping methods during the capture of complex traits like leaf inclination angles and internal fruit structures (Jiang et al., 2024; Zhu et al., 2022). Moreover, RGB image-based phenotyping methods are substantial due to attributes such as low cost, non-destructiveness, high accuracy, and real-time data acquisition when compared to other expensive sensing systems, including hyperspectral, fluorescence, and LiDAR systems (Johansen et al., 2020; Jollet et al., 2023). However, despite these advantages, some limitations of RGB-based phenotyping are noticeable. One major limitation is image quality, which depends heavily on light conditions, shadows, and background impurities (Hobart et al., 2020). The threshold-based image segmentation techniques confine this issue to some extent by separating objects from the background in an effective way. However, the higher computational power required is a significant issue compared with other methods. The leaf overlapping is another significant problem that has been solved by deep imaging and 3D imaging strategies. RGB images always give information about physical conditions, and measurements are highly variable; therefore, these techniques are not perfect for determining the internal and composition-based indices. Another noticeable issue is the

low stability of airborne phenotyping systems which, leads to low-accuracy data in open-field and large-scale studies (Fu et al., 2021). Although machine learning approaches have improved image segmentation, challenges with segmentation, predominantly exist under varied field conditions (Kolhar & Jagtap, 2021; Tan et al., 2024). Threshold-based segmentation algorithms perform effectively under varying light circumstances; yet, they necessitate increased computational time due to the several stages involved in threshold adjustment. Machine learning-based methodologies exhibit excellent accuracy; yet they are intricate due to the need for many training phases for precise segmentation. Dense canopies or overlapping plant components, particularly during image acquisition, may inhibit identifying underlying structures such as fruits or tillers (Diago et al., 2012; Feldmann et al., 2020). Furthermore, the narrow wavelength range of RGB sensors limits the accuracy of water content estimation, pigment composition analysis, and early stress detection compared to multispectral or thermal imaging sensors (Guo et al., 2021; Musse et al., 2021). Nevertheless, RGB imaging provides an effective and accessible alternative for high-throughput phenotyping, particularly when combined with robust computational pipelines, especially in resource-limited environments.

Conclusions and Future Direction

This review evaluates the applications, advantages, and limitations of various RGB imaging techniques used in plant phenotyping. Image-based phenotyping methods have become essential in plant breeding, crop management, and the quality evaluation of commercially valuable crops. Given the crucial factors affecting global food security, the expedited breeding cycles of new crop varieties have emerged as a paramount objective worldwide. This study highlights RGB image-based approaches and their applications in phenotyping, emphasizing the relatively accessible and cost-effective platforms predominantly used in current high-throughput phenotyping methods. This technique has the potential to determine the morphological traits with higher accuracy. However, RGB images are less sensitive in providing physiological data. The following challenges in RGB image-based plant phenotyping, including light conditions, varied backgrounds, overlapping leaves, and the circadian movement of plant leaves, have a substantial impact on accuracy. To address the limitations in RGB image-based plant phenotyping and enhance the accuracy of existing systems, future studies should be focused on precise image acquisition systems, image processing (image segmentation), identifying novel color parameters, the advancement of complicated analytical methodologies (artificial intelligence and robust machine learning models), and integration with complementary sensor technologies. Moreover, these techniques should focus on collaborating, optimizing, and identifying the most precise and accurate conditions for the high-throughput phenotyping process, considering both instrumental (imaging setup) and algorithm-based (image processing) factors.

Declarations

Ethical Approval Certificate

There is no content in the study that requires ethics committee approval.

Author Contribution Statement

M.V.T.T wrote the original draft, and K.M.K. and R.L.R reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

References

Alaguero-Cordovilla, A., Gran-Gómez, F., Tormos-Moltó, S., & Pérez-Pérez, J. (2018). Morphological Characterization of Root System Architecture in Diverse Tomato Genotypes during Early Growth. *International Journal of Molecular Sciences*, *19*(12), 3888. https://doi.org/10.3390/ijms19123888

Araus, J. L., Kefauver, S. C., ZamanAllah, M., Olsen, M. S., & Cairns, J. E. (2018). Translating highthroughput phenotyping into genetic gain. *Trends in Plant Science*, *23*(5), 451–466.

Brainard, S. H., Bustamante, J. A., Dawson, J. C., Spalding, E. P., & Goldman, I. L. (2021). A Digital Image-Based Phenotyping Platform for Analyzing Root Shape Attributes in Carrot. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.690031

Cabrera-Bosquet, L., Crossa, J., Zitzewitz, von, Serret, M. D., & Araus, L. (2012). High-throughput phenotyping and genomic selection: The frontiers of crop breeding converge F. *Journal of Integrative Plant Biology*, *54*(5), 312–320.

Cen, H., Wan, L., Zhu, J., Li, Y., Li, X., Zhu, Y., Weng, H., Wu, W., Yin, W., & Xu, C. (2019). Dynamic monitoring of biomass of rice under different nitrogen treatments using a lightweight UAV with dual imageframe snapshot cameras. *Plant Methods*, *15*, 1–16.

Chen, Y., Chao, K., & Kim, M. S. (2002). Machine vision technology for agricultural applications. *Computers and Electronics in Agriculture*, *36*(23), 173–191.

Darrigues, A., Hall, J., van, Francis, D. M., Dujmovic, N., & Gray, S. (2008). Tomato analyzercolor test: a new tool for efficient digital phenotyping. *Journal of the American Society for Horticultural Science*, *133*(4), 579–586.

Davies, E. R. (2012). *Computer and machine vision: theory, algorithms, practicalities*. Academic Press.

Deery, D., Jimenez-Berni, J., Jones, H., Sirault, X., & Furbank, R. (2014). Proximal Remote Sensing Buggies and Potential Applications for Field-Based Phenotyping. *Agronomy*, *4*(3), 349–379. https://doi.org/10.3390/agronomy4030349

Dellen, B., Scharr, H., & Torras, C. (2015). Growth Signatures of Rosette Plants from Time-Lapse Video. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, *12*(6), 1470–1478. https://doi.org/10.1109/TCBB.2015.2404810

Diago, M.-P., Correa, C., Millán, B., Barreiro, P., Valero, C., & Tardaguila, J. (2012). Grapevine Yield and Leaf Area Estimation Using Supervised Classification Methodology on RGB Images Taken under Field Conditions. *Sensors*, *12*(12), 16988–17006. https://doi.org/10.3390/s121216988

Fahlgren, N., Gehan, M. A., & Baxter, I. (2015). Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Current Opinion in Plant Biology*, *24*, 93–99. https://doi.org/10.1016/j.pbi.2015.02.006

FAO. (2024). Agricultural Production Statistics 2010–2023. *FAOSTAT Analytical Briefs, No. 96.*

Fawzy, E., Harb, A., Allam, D. G., & Abdelraouf, E. (2022). Thermal and RGB imaging as potential tools for assessing chlorophyll and nutritional performance of pepper (Capsicum annum). *Journal of Agricultural and Environmental Sciences*, *21*(3), 80–100.

Feldmann, M. J., Hardigan, M. A., Famula, R. A., López, C. M., Tabb, A., Cole, G. S., & Knapp, S. J. (2020). Multi-dimensional machine learning approaches for fruit shape phenotyping in strawberry. *GigaScience*, *9*(5). https://doi.org/10.1093/gigascience/giaa030

Feng, H., Tao, H., Li, Z., Yang, G., & Zhao, C. (2022). Comparison of UAV RGB imagery and hyperspectral remote sensing data for monitoring winter wheat growth. *Remote Sensing*, *14*(15), 3811.

Fiorani, F., & Schurr, U. (2013). Future scenarios for plant phenotyping. *Annual Review of Plant Biology*, *64*(1), 267–291.

Fu, X., Bai, Y., Zhou, J., Zhang, H., & Xian, J. (2021). A method for obtaining field wheat freezing injury phenotype based on RGB camera and software control. *Plant Methods*, *17*(1). https://doi.org/10.1186/s13007-021-00821-7

Gang, M.-S., Kim, H.-J., & Kim, D.-W. (2022). Estimation of Greenhouse Lettuce Growth Indices Based on a Two-Stage CNN Using RGB-D Images. *Sensors*, *22*(15), 5499. https://doi.org/10.3390/s22155499

Ge, Y., Bai, G., Stoerger, V., & Schnable, J. C. (2016). Temporal dynamics of maize plant growth, water use, and leaf water content using automated high throughput RGB and hyperspectral imaging. *Computers and Electronics in Agriculture*, *127*, 625–632. https://doi.org/10.1016/j.compag.2016.07.028

Gill, T., Gill, S. K., Saini, D. K., Chopra, Y., de Koff, J. P., & Sandhu, K. S. (2022). A Comprehensive Review of High Throughput Phenotyping and Machine Learning for Plant Stress Phenotyping. *Phenomics*. https://doi.org/10.1007/s43657-022-00048-z

Granier, C., L. Aguirrezabal, Chenu, K., Sarah Jane Cookson, Dauzat, M., Philippe Hamard, Thioux, J. J., Rolland, G., Sandrine Bouchier-Combaud, Lebaudy, A., Muller, B., Thierry Simonneau, & François Tardieu. (2006). PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytologist*, *169*(3), 623–635. https://doi.org/10.1111/j.1469-8137.2005.01609.x

Guo, Y., Chen, S., Wu, Z., Wang, S., Robin Bryant, C., Senthilnath, J., Cunha, M., & Fu, Y. H. (2021). Integrating Spectral and Textural Information for Monitoring the Growth of Pear Trees Using Optical Images from the UAV Platform. *Remote Sensing*, *13*(9), 1795. https://doi.org/10.3390/rs13091795

Hobart, M., Pflanz, M., Weltzien, C., & Schirrmann, M. (2020). Growth Height Determination of Tree Walls for Precise Monitoring in Apple Fruit Production Using UAV Photogrammetry. *Remote Sensing*, *12*(10), 1656. https://doi.org/10.3390/rs12101656

Holland, K. H., Lamb, D. W., & Schepers, J. S. (2012). Radiometry of proximal active optical sensors (AOS) for agricultural sensing. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, *5*(6), 1793–1802.

Horgan, G. W., Song, Y., Glasbey, C. A., van der Heijden, G. W. A. M., Polder, G., Dieleman, J. A., Bink, M. C. A. M., & van Eeuwijk, F. A. (2015). Automated estimation of leaf area development in sweet pepper plants from image analysis. *Functional Plant Biology*, *42*(5), 486. https://doi.org/10.1071/fp14070

Houle, D., Govindaraju, Diddahally R, & Omholt, S. (2010). Phenomics: the next challenge. *Nature Reviews Genetics*, *11*(12), 855–866.

Jia, L., Buerkert, A., Chen, X., Roemheld, V., & Zhang, F. (2004). Low-altitude aerial photography for optimum N fertilization of winter wheat on the North China Plain. *Field Crops Research*, *89*(2-3), 389–395. https://doi.org/10.1016/j.fcr.2004.02.014

Jiang, S., Wu, X., Wang, Q., Pei, Z., Wang, Y., Jin, J., Guo, Y., Song, R., Zang, L., Liu, Y.-J., & Hao, G. (2024). Auto-LIA: The Automated Vision-Based Leaf Inclination Angle Measurement System Improves Monitoring of Plant Physiology. *Plant Phenomics*, *6*, 0245. https://doi.org/10.34133/plantphenomics.0245

Jiang, Y., & Yang, Y. (2022). High-throughput phenotyping for plant growth and biomass yield of switch grass under a controlled environment. *Grass Research*, *2*(1), 1–7. https://doi.org/10.48130/gr-2022-0004

Johansen, K., Morton, M. J. L., Malbeteau, Y., Aragon, B., Al-Mashharawi, S., Ziliani, M. G., Angel, Y., Fiene, G., Negrão, S., Mousa, M. A. A., Tester, M. A., & McCabe, M. F. (2020). Predicting Biomass and Yield in a Tomato Phenotyping Experiment Using UAV Imagery and Random Forest. *Frontiers in Artificial Intelligence*, *3*(28). https://doi.org/10.3389/frai.2020.00028

Jollet, D., Junker-Frohn, L. V., Steier, A., Meyer-Lüpken, T., & Müller-Linow, M. (2023). A new computer vision workflow to assess yield quality traits in bush bean (Phaseolus vulgaris L.). *Smart Agricultural Technology*, *5*, 100306–100306. https://doi.org/10.1016/j.atech.2023.100306

Kim, D.-W., Yun, H. S., Jeong, S.-J., Kwon, Y.-S., Kim, S.-G., Lee, W. S., & Kim, H.-J. (2018). Modeling and Testing of Growth Status for Chinese Cabbage and White Radish with UAV-Based RGB Imagery. *Remote Sensing*, *10*(4), 563. https://doi.org/10.3390/rs10040563

Kim, J., & Chung, Y. S. (2021). A short review of RGB sensor applications for accessible high-throughput phenotyping. *Journal of Crop Science and Biotechnology*, *24*(5), 495–499. https://doi.org/10.1007/s12892-021-00104-6

Kior, A., Yudina, L., Zolin, Y., Sukhov, V., & Sukhova, E. (2024). RGB imaging as a tool for remote sensing of characteristics of terrestrial plants: A review. *Plants*, *13*(9), 1262.

Kolhar, S., & Jagtap, J. (2021). Plant trait estimation and classification studies in plant phenotyping using machine vision – A review. *Information Processing in Agriculture*, *10*(1). https://doi.org/10.1016/j.inpa.2021.02.006

Li, L., Zhang, Q., & Huang, D. (2014). A Review of Imaging Techniques for Plant Phenotyping. *Sensors*, *14*(11), 20078–20111. https://doi.org/10.3390/s141120078

Liming, X., & Yanchao, Z. (2010). Automated strawberry grading system based on image processing. *Computers and Electronics in Agriculture*, *71*, S32–S39.

Liu, Y., Chen, Y., Wen, M., Lu, Y., & Ma, F. (2023). Accuracy comparison of estimation on cotton leaf and plant nitrogen content based on UAV digital image under different nutrition treatments. *Agronomy*, *13*(7), 1686.

Liu, Y., Hatou, K., Aihara, T., Kurose, S., Akiyama, T., Kohno, Y., Lu, S., & Omasa, K. (2021). A robust vegetation index based on different UAV RGB images to estimate SPAD values of naked barley leaves. *Remote Sensing*, *13*(4), 686.

Luana, S., de, Valentim, D. A., Andrade, M. T., Conti, L., & Rossi, G. (2020). Determining the leaf area index and percentage of area covered by coffee crops using UAV RGB images. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, *13*, 6401–6409.

Ma, Z., Rayhana, R., Feng, K., Liu, Z., Xiao, G., Ruan, Y., & Sangha, J. S. (2022). A review on sensing technologies for high throughput plant phenotyping. *IEEE Open Journal of Instrumentation and Measurement*, *1*, 1–21.

Mahlein, A. (2016). Plant disease detection by imaging sensors–parallels and specific demands for precision agriculture and plant phenotyping. *Plant Disease*, *100*(2), 241–251.

Mahlein, A.-K., Steiner, U., Hillnhütter, C., Dehne, H.-W., & Oerke, E.-C. (2012). Hyperspectral imaging for small-scale analysis of symptoms caused by different sugar beet diseases. Plant Methods, 8(1), 3. https://doi.org/10.1186/1746-4811-8-3

Mansoor, S., Karunathilake, E. M. B. M., Tuan, T. T., & Chung, Y. S. (2024). Genomics, Phenomics, and Machine Learning in Transforming Plant Research: Advancements and Challenges. *Horticultural Plant Journal*. https://doi.org/10.1016/j.hpj.2023.09.005

Minaee, S., Boykov, Y., Porikli, F., Plaza, A., Kehtarnavaz, N., & Terzopoulos, D. (2021). Image segmentation using deep learning: A survey. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, *44*(7), 3523–3542.

Mir, R. R., Reynolds, M., Pinto, F., Khan, M. A., & Bhat, M. A. (2019). High throughput phenotyping for crop improvement in the genomics era. *Plant Science*, *282*, 60–72.

Montes, J. M., Melchinger, Albrecht E, & Reif, J. C. (2007). Novel throughput phenotyping platforms in plant genetic studies. *Trends in Plant Science*, *12*(10), 433–436.

Musse, M., Hajjar, G., Ali, N., Billiot, B., Joly, G., Jérémy Pépin, Stéphane Quellec, Sylvain Challois, Mariette, F., Cambert, M., Fontaine, C., Ngo-Dinh, C., Jamois, F., Barbary, A., Leconte, P., Deleu, C., & Leport, L. (2021). A global non-invasive methodology for the phenotyping of potato under water deficit conditions using imaging, physiological and molecular tools. *Plant Methods*, *17*(1). https://doi.org/10.1186/s13007-021-00771-0

Omari, M. K., Lee, J., Faqeerzada, M. A., Joshi, R., Park, E., & Cho, B. (2020). Digital image based plant phenotyping: a review. *Korean Journal of Agricultural Science*, *47*(1), 119–130.

Qiu, R., Wei, S., Zhang, M., Li, H., Sun, H., Liu, G., & Li, M. (2018). Sensors for measuring plant phenotyping: A review. *Int J Agric & Biol Eng*, *11*(2), 1–17.

Rasmussen, J., Ntakos, G., Nielsen, J., Svensgaard, J., Poulsen, R. N., & Christensen, S. (2016). Are vegetation indices derived from consumer grade cameras mounted on UAVs sufficiently reliable for assessing experimental plots? *European Journal of Agronomy*, *74*, 75–92.

Rehman, T. U., Ma, D., Wang, L., Zhang, L., & Jin, J. (2020). Predictive spectral analysis using an end-to-end deep model from hyperspectral images for high-throughput plant phenotyping. *Computers and Electronics in Agriculture*, *177*, 105713. https://doi.org/10.1016/j.compag.2020.105713

Rezzouk, F. Z., Gracia Romero, A., Kefauver, S. C., Gutiérrez, N. A., Aranjuelo, I., Serret, M. D., & Luis, A. J. (2020). Remote sensing techniques and stable isotopes as phenotyping tools to assess wheat yield performance: Effects of growing temperature and vernalization. *Plant Science*, *295*, 110281.

Rocha, R., Fernando, D., Bandeira, M., Alves, L., & Jorge. (2022). Image-based phenotyping of cassava roots for diversity studies and carotenoids prediction. *PLOS ONE*, *17*(1), e0263326–e0263326. https://doi.org/10.1371/journal.pone.0263326

Sie, E. K., Oteng-Frimpong, R., Kassim, Y. B., Puozaa, D. K., Adjebeng-Danquah, J., Masawudu, A. R., ... & Balota, M. (2022). RGB-image method enables indirect selection for leaf spot resistance and yield estimation in a groundnut breeding program in Western Africa. *Frontiers in Plant Science*, *13*, 957061.

Singh, H., Kumar, N., & Kumar, A. (2024). Enhancing Resource Use Efficiency in Crops Through Plant Functional Traits. *Plant Functional Traits for Improving Productivity. Springer, Singapore*, 97–117. https://doi.org/10.1007/978-981-97-1510-7_6

Tan, G. D., Chaudhuri, U., Varela, S., Ahuja, N., & Leakey, A. D. B. (2024). Machine learning-enabled computer vision for plant phenotyping: a primer on AI/ML and case study on stomatal patterning. *Journal of Experimental Botany*. https://doi.org/10.1093/jxb/erae395

Tharuka, M. V. T., & Wijerathne, H. P. B. (2024). Development of an image-based alternative method for soil plant analysis development (SPAD) reading. *Young Scientists' Conference on Multidisciplinary Research-2024*, 1.

Van Dusschoten, D., Metzner, R., Kochs, J., Postma, J. A., Pflugfelder, D., Bühler, J., Schurr, U., & Jahnke, S. (2016). Quantitative 3D Analysis of Plant Roots Growing in Soil Using Magnetic Resonance Imaging. *Plant Physiology*, *170*(3), 1176–1188. https://doi.org/10.1104/pp.15.01388

Van, Burchfield, D. R., Witt, T. D., Price, K. P., & Sharda, A. (2020). Drones in agriculture. *Advances in Agronomy*, *162*, 1–30.

Walter, A., Finger, R., Huber, R., & Buchmann, N. (2017). Opinion: Smart farming is key to developing sustainable agriculture. *Proceedings of the National Academy of Sciences*, *114*(24), 6148–6150. https://doi.org/10.1073/pnas.1707462114

Walter, A., Liebisch, F., & Hund, A. (2015). Plant phenotyping: from bean weighing to image analysis. *Plant Methods*, *11*(1), 14. https://doi.org/10.1186/s13007-015-0056-8

Walter, J., Edwards, J., Cai, J., McDonald, G., Miklavcic, S. J., & Kuchel, H. (2019). High-throughput field imaging and basic image analysis in a wheat breeding programme. *Frontiers in plant science*, *10*, 449.

Wasonga, D. O., Yaw, A., Kleemola, J., Alakukku, L., & Mäkelä, P. S. A. (2021). Red-Green-Blue and Multispectral Imaging as Potential Tools for Estimating Growth and Nutritional Performance of Cassava under Deficit Irrigation and Potassium Fertigation. *Remote Sensing*, *13*(4), 598. https://doi.org/10.3390/rs13040598

Widjaja Putra, B. T., & Soni, P. (2017). Enhanced broadband greenness in assessing Chlorophyll a and b, Carotenoid, and Nitrogen in Robusta coffee plantations using a digital camera. *Precision Agriculture*, *19*(2), 238–256. https://doi.org/10.1007/s11119-017-9513-x

Xiao, Q., Bai, X., Zhang, C., & He, Y. (2021). Advanced high-throughput plant phenotyping techniques for genome-wide association studies: A review. *Journal of Advanced Research*, *35*, 215–230. https://doi.org/10.1016/j.jare.2021.05.002

Yadav, S. P., Ibaraki, Y., & Dutta Gupta, S. (2009). Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *100*(2), 183–188. https://doi.org/10.1007/s11240-009-9635-6

Yonis, B. O., Pino del Carpio, D., Wolfe, M., Jannink, J.-L., Kulakow, P., & Rabbi, I. (2020). Improving root characterisation for genomic prediction in cassava. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-64963-9

Yu, L., Shi, J., Huang, C., Duan, L., Wu, D., Fu, D., Wu, C., Xiong, L., Yang, W., & Liu, Q. (2020). An integrated rice panicle phenotyping method based on X-ray and RGB scanning and deep learning. *The Crop Journal*, *9*(1), 42–56. https://doi.org/10.1016/j.cj.2020.06.009

Zhu, Y., Gu, Q., Zhao, Y., Wan, H., Wang, R., Zhang, X., & Cheng, Y. (2022). Quantitative Extraction and Evaluation of Tomato Fruit Phenotypes Based on Image Recognition. *Frontiers in Plant Science*, *13*, 859290. https://doi.org/10.3389/fpls.2022.859290