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# Study of Phenotype Analysis of Maize Plant: Hyperspectral Image Perspective

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**Abstract:** *Maize, alongside rice and wheat, constitutes a trio of crops responsible for over 50% of the global calorie consumption. To address the rising food demand, enhancing the productivity and stress resilience of these crops becomes imperative. However, the progress of plant breeding initiatives is hindered by the cost and time constraints associated with acquiring plant phenotype data. To overcome these limitations and advance the field, there is a need for datasets that connect new forms of high-throughput phenotype data, gathered from plants, to the performance of identical genotypes in diverse agronomic settings and habitats. These datasets will pave the way for the development of innovative statistical and Machine Learning techniques, empowering researchers to expedite crop improvement efforts effectively.*

**Keywords:** *Maize, Image, Classification, regression*

## I. INTRODUCTION

The green revolution helped lessen hunger and drought across the world in the 1960s and 1970s even while population growth accelerated. It did this by increasing the yields of numerous significant crops. The development of new major grain crop varieties with increased yield potential through traditional phenotypic selection was a crucial aspect of the green revolution. Since then, the demand for food has consistently grown, leading to significant endeavours in the public and private sectors to cultivate crop types with even higher yield potential. As the easily achievable advancements in productivity diminish, achieving further improvements in yield requires greater dedication of effort and resources. Latest research has found that harvest gains for some key grain crops have slowed or stopped in significant parts of the world. If the agricultural output is to keep growing to fulfil the requirements of a rising global population, new approaches to plant breeding must be devised.

Phenotype is a critical bottleneck in current plant breeding. There are two ways to use phenotyping. Initially, a plant breeder can identify lines with the highest yield potential and stress tolerance in a particular environment by extensively phenotyping a large number of lines. Second, enough detailed phenotyping measures from enough diverse plants, along with genotypic data, can be used to identify sections of a plant species' genome that contain advantageous or harmful alleles. After that, the breeder can generate new crop kinds with as many advantageous alleles as feasible while removing as many negative alleles as possible.

Phenotyping proves to be both time-consuming and costly. As breeders strive to uncover numerous alleles, each with a minor impact, the demand for phenotyping increases to achieve a specific rise in yield potential. However, plant phenotyping techniques integrated with high-throughput Machine Learning offer a promising solution to this bottleneck. These advanced tools allow precise quantification of even the smallest plant features, and their unit costs are likely to decrease with scale. In contrast, traditional phenotyping remains labor-intensive and does not benefit from the same cost reductions.

In several recent pilot experiments, various image-processing algorithms were employed to obtain phenotypic measurements from crop plants. Among the computer vision-based plant phenotyping approaches, the RGB (Red, Green, Blue) camera technology was predominantly used, as it is commonly found in the consumer market. Additionally, fluorescence and near-infrared (NIR) cameras have been utilized in high-throughput plant phenotyping endeavors, particularly when studying the response of plants to different abiotic stress conditions.

## II. LITERATURE SURVEY

The article "HTPheno: an image analysis pipeline for high-throughput plant phenotyping," published in BMC Bioinformatics in 2011 <sup>[1]</sup>, introduces HTPheno, an image analysis pipeline designed for high-throughput plant phenotyping. The pipeline enables automated and efficient analysis of large-scale plant image datasets, allowing researchers to extract and quantify various phenotypic traits for plants in high-throughput experiments. The authors utilized the ImageJ plugin multi Otsu threshold, which implements the Otsu threshold algorithm to find up to 5 optimal threshold levels for an image.

However, this approach did not provide the necessary thresholds. As an alternative, the authors employed a pixel-based segmentation technique called multidimensional histogram thresholding (MHT) to address this issue effectively<sup>[1]</sup>.

Christopher N. Topp et al.<sup>[2]</sup> and Zurek PR et al. (2015)<sup>[3]</sup> conducted 3D phenotyping and quantitative trait locus (QTL) mapping to identify essential regions of the rice genome that control root architecture. The study focused on analyzing root traits in three dimensions and determining the genetic loci responsible for regulating root development in rice. The study<sup>[2]</sup> employed multivariate QTL analysis to identify the central region controlling root system architecture (RSA) growth. They utilized PCA and examined QTL clusters on GiA roots. The paper's statistical analysis included univariate and multivariate QTL analysis, utilizing DFA to identify traits. Additionally, the authors applied adaptive thresholding to the dataset. It has some potential limitations include sample size, genetic diversity, and environmental variability. In<sup>[3]</sup> they adopt gel-based phenotyping system for maize, they implemented various modifications to account for the larger size and faster growth rate of maize roots.

In their 2019 research, Zheng et al.<sup>[4]</sup> investigated the "Shared Genetic Control of Root System Architecture between Zea mays and Sorghum bicolor." While Arabidopsis has been extensively studied to understand the genetic regulation of root development, connecting markers with phenotypic variance allows genome-wide association studies (GWAS)<sup>[4]</sup> to uncover genes influencing quantitative trait variation. To facilitate genetic mapping, the researchers developed several root phenotyping pipelines.

Xuehai Zhang et al. (2017)<sup>[7]</sup> utilized the Otsu method for extracting components, followed by a region growing algorithm to obtain the entire plant's binary image and histogram texture traits were computed by matching the binary and original color images. The autor used open source database which is available on <http://plantphenomics.hzau.edu>. The evaluation was done using MAPE and SDAPE values for identification of the leaf skeleton, Hough transformation was they applied<sup>[7]</sup>. Stem length and total leaf measurements were derived from this information. Each leaf skeleton was labeled, and features like leaf angle, length, and curvature were determined for each leaf. Growth-related features were calculated using biomass (fresh and dried weights) obtained at different time points.

Dijun Chen et al.<sup>[19]</sup> aimed to understand the phenotypic components of crop plant growth and their responses to drought stress using high-throughput image analysis. They employed clustering (PCA), classification (SVM), and regression (Mean Square Error for Regression) to non-destructively capture and analyse various growth-related traits of crop plants under both normal growth conditions and drought stress. The study involved the use of sophisticated image analysis tools to quantify and assess plant phenotypes, such as shoot and root growth, leaf morphology, and developmental patterns. The researchers collected a large amount of data through this high-throughput imaging approach which is available in this website: <http://iap.ipk-pgatersleben.de/modeling>. By analysing the data, the authors were able to dissect the complex traits contributing to crop plant growth and their responses to drought. This allowed them to identify specific phenotypic components that play crucial roles in drought tolerance and growth adaptation in crop plants Understanding these phenotypic components can potentially help breeders and researchers develop more drought-resistant and resilient crop varieties, contributing to food security in the face of changing climate conditions. Overall, this research highlights the significance of high-throughput image analysis in unravelling the complexity of plant phenotypes and their responses to environmental challenges, ultimately aiding in crop improvement efforts.

The article "Deep phenotyping: deep learning for temporal phenotype/genotype classification"<sup>[11]</sup> explores the use of deep learning techniques for classifying plant phenotypes and genotypes over time. The research focuses on "deep phenotyping," that utilizing advanced deep learning algorithms to analyse and classify temporal plant trait data. They achieved notable accuracy (76.8 - 93%) by applying deep feature extraction for CNN parameter training using the SGD (Stochastic Gradient Descent) method. Additionally, they employed Otsu's method and SVM classifier for binary classification, and RNN model to analyze visual information. The study highlights the potential of deep learning methods in understanding plant biology and advancing crop research.

Zhikai Liang et al.<sup>[17]</sup> employed two main methodologies: conventional time-series imaging and hyperspectral time-series imaging. First one is Conventional Time-Series Imaging: This involves capturing regular images of the maize plants over time using conventional RGB cameras. These images are then used to analyze plant growth and monitor phenotypic changes throughout the plant's development. The second one is Hyperspectral Time-Series Imaging: It uses cameras that capture a wide range of spectral bands beyond the visible spectrum. This allows for more detailed and specific measurements of plant traits, such as detecting stress responses or assessing biochemical changes over time. The study utilized various techniques for analysing the plant region of interest, including threshold NDVI (Normalized Difference Vegetation Index) and PCA (Principal Component Analysis)<sup>[17]</sup>. Biomass was predicted using MARS and SVM models. The research also employed a linear regression model on Giga science database which actually available on this "Gigasciencedatabase.gigaDB" website which assess genotype effect.

In their 2019 study, Yanjun Su et al.<sup>[14]</sup> evaluated maize phenotype dynamics under drought stress using terrestrial lidar. They collected lidar data at six growth phases for 20 maize types under drought stress using a terrestrial laser scanner.

The lidar point clouds were utilized to assess three drought-related phenotypes at the individual plant level: plant height, plant area index (PAI), and predicted leaf area (PLA). The authors used the Regression technique with RMSE for their analyses and get results demonstrated that terrestrial lidar data could accurately estimate plant height (96%), PLA (92%), and PAI (70%)<sup>[14]</sup>. The phenotypes exhibited a trend of increasing and then decreasing during the growth phase.

The paper "Digital Imaging of Root Traits (DIRT): a high-throughput computing and collaboration platform for field-based root phenomics,"<sup>[15]</sup> published in *Plant Methods* in 2015, introduces DIRT, a platform designed for high-throughput computing and collaboration in field-based root phenomics that freely accessible on <http://dirt.iplantcollaborative.org>. DIRT utilizes digital imaging to analyze root traits, enabling efficient and collaborative research on root phenotypes in field environments. The platform provides researchers with a powerful tool to study root characteristics on a large scale, leading to valuable insights into plant root systems and their responses to different environmental conditions. The authors opted for Python as the programming language to develop the pipeline for estimating the Root System Architecture (RSA) trait.

In "Leaf segmentation in plant phenotyping: a collation study" by Hanno Scharr et al. (2016)<sup>[16]</sup> focuses on advancing leaf segmentation methods for plant phenotyping. They bring together various techniques, including unsupervised clustering using SLIC, IPk pipeline, and Nottingham segmentation with SLIC super pixel, to accurately segment leaves from plant images. The study aims to provide valuable insights into effective leaf segmentation approaches, essential for automated analysis and understanding of plant traits. Using publicly available datasets, their unsupervised approach achieved segmentation accuracy above 90%

Patricio Grassini et al. (2013)<sup>[18]</sup> investigates historical crop production trends to differentiate between yield advances and yield plateaus. The research aims to understand whether agricultural yield improvements have reached a plateau or continue to advance over time. The study provides valuable insights into the long-term productivity trends in crop production, which is crucial for sustainable food production and agricultural planning. In this study, the authors assessed each crop-region model combination by calculating the Root Mean Square Error (RMSE) and used the best-fit-model, namely PW and LUP or LLP, for parameter estimation. They utilized an open-source dataset obtained from <http://faostat.fao.org/> for their research.

Sruti Das Choudhury et al. (2016)<sup>[9]</sup> Paper on "Automated vegetative stage phenotyping analysis of maize plants using visible light images DS-FEW". In this paper Author use techniques for binarizing the extracted foreground using Otsu's thresholding. They introduce a new derived holistic phenotype, namely, bi-angular convex-hull area ratio (BA<sub>chR</sub>)

$$BA_{chR} = \frac{\text{AreaCH at side view } 0}{\text{AreaCH at side view } 90}$$

Plant Aspect Ration (PAR) that integrates data on plant height and leaf extent [9].

$$PAR = \frac{\text{HeightBR at side view}}{\text{Diameter MEC at top side view}}$$

For this experiment dataset are Panicoid Phenomap-1.

Yufeng Ge et al. (2016)<sup>[20]</sup> research on "Temporal dynamics of maize plant growth, water use, and leaf water content using automated high throughput RGB and hyperspectral imaging". Author use techniques Normalized and Regression, for normalized NDVI and for regression PLSR. The author use PLSR for the extracted plant leaf reflectance spectra for predicted Leaf Water Content (LWC). For these Techniques, Author use a freely available dataset for the prediction of LWC.

Noah Fahlgren et al. (2015)<sup>[21]</sup> paper on "A Versatile Phenotyping System and Analytics Platform Reveals Diverse Temporal Responses to Water Availability in Seteria". In this paper Phenotype extraction using PlantCV software. Integrated Analysis Platform (IAP) Analysis larger model. Techniques are used i.e. non – linear least square regression was used to estimate the growth parameter. The author use normalization using PCA and Principal Component regression on <https://www.lemnatec.com.dataset>. Here predicted manual tiller Ordinary least square regression was used to generate a model for the number of tillers: TC = M<sub>tw</sub> – HW + tiller number

Pedro Andrade-Sanchez et al. (2014)<sup>[6]</sup> "Development and evaluation of a field-based high-throughput phenotyping platform". The author uses Techniques i.e. normalize using Normalize Difference Vegetation Index (NDVI). Root Mean Square Error (RMSE). In statistical analysis following formula is used for variance component estimates from each final model was used to estimate H<sup>2</sup>.

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_e^2)_r} = \frac{\sigma_g^2}{\sigma_p^2}$$

Nadia AI-Tamimi et al. (2016)<sup>[22]</sup> "Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping". High – Through Phenotype analysis of plant growth rate and transpiration Use Efficiency (TUE) are analyzed using a new association model. Mixed Linear model (MLM) analysis was performed AGR, RGRs, TRs, and TUEs for estimation Restricted Maximum Likelihood Estimation (REML).

According to the following equation TR was calculated from the transpiration and evaporation, for each plant and each day. For High-Throughput Phenotype analysis use the dataset on the website <https://datadryad.org/dio:10.5061/dryad.3118j>.

$$TR_{(tk-1) \rightarrow tk} = \frac{T(tk-1,tk) - 0}{tk - tk-1} = \frac{(WA-WB) - E}{tk - tk-1}$$

From the following formula spatial correction of phenotype analysis, the maximal MLM for this analysis was calculated:  $y = X\beta + Zu + e$

According to the following equation, Genome-wide association analysis is the second approach, the MLM referred to as the interaction model,  $y = X\beta + S\alpha + T\gamma + Zu + e$

Dijun Chen et al. (2018)<sup>[23]</sup> “Predicting plant biomass accumulation from image-derived parameters”. In this paper Predicted Plant Biomass using Linear Regression Models i.e. Multivariate linear Regression (MLR), Multivariate Linear Regression Splines(MARS), Random Forest(RF), and Support Vector Regression (SVR) for these regression models. To evaluate the performance of predictive models, The predictive accuracy of the model can be measured by,

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

The Root mean squared relative error of cross-validation is defined as,

$$RMSRE = \sqrt{\sum_{i=1}^n \left(\frac{y_i - \hat{y}_i}{y_i}\right)^2} \div S$$

Predictive power was reflected by bias  $\mu$  between the predicted and observed values,

$$\mu = \frac{1}{n} \sum_{i=1}^n \frac{\hat{y}_i - y_i}{y_i}$$

Malachy T. Campbell et al. (2015)<sup>[24]</sup> “Integrating Image-Based Phenomics and Association Analysis to Dissect the Genetic Architecture of Temporal Salinity Responses in Rice”. This paper for Analysis of data using hierarchical Clustering Analysis and Pearson correlation Analysis. Image Acquisition and image processing techniques are applied. For this dataset are available on these website <https://mirrors.iplantcollaborative.org>.

LEO BREIMAN et al. (2001)<sup>[25]</sup> “Random Forest”. In this paper Random forest using random input selection and prediction error in random forest. Author uses Random Forest for regression, classification got 90% accuracy.

Dominik K. Großkinsky et al. (2015)<sup>[12]</sup> “Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype knowledge gap”. The author use image processing techniques for predicting genotype and phenotype.

Nora Honsdorf et al. (2014)<sup>[27]</sup> “High-Throughput Phenotyping to Detect Drought Tolerance QTL in Wild Barley Introgression Lines”. In this paper mixed model analysis a Dunnett test was conducted where least square means(LSMEANS). They also use Mixed model Analysis of variance. Statistical analyses were performed Heritabilities across the treatment were calculated as

$$h^2 = V_G / [V_G + V_{GT/t} + V_{GE/e} + V_{GET/et} + V_{R/etr}]$$

Analysis of variance was carried out with the procedure MIXED using model 1.

Model 1 :

$$Y_{ikl} = \mu + L_i + T_j + E_k + L \times T_{ij} + L \times E_{ik} + B(E \times T_{kj})_l + \epsilon_{ijkl}$$

Model 2 :

$$Y_{ikl} = \mu + L_i + E_k + L \times E_{ik} + B(E_k)_l + \epsilon_{ijkl}$$

For these freely available dataset i.e. Panicoid phenomap1

### III. DATASET

Researchers commonly utilize the Panicoid phenome-1 dataset for phenotype analysis due to its comprehensive data, including RGB, Hyperspectral, Fluorescence, and Thermal Infrared images. This dataset is invaluable for conducting phenotyping research, specifically in areas like leaf counting, leaf alignment, leaf segmentation, leaf tracking, and 3D leaf reconstruction. It has become the standard dataset for such studies.

The Panicoid phenome-1 dataset consists of a substantial 485 GB of images, making it an excellent resource for evaluating the performance of proposed algorithms. To support and ensure consistent comparisons, we present the University of Nebraska-Lincoln Panicoid phenome-1 dataset, along with corresponding ground truth data.

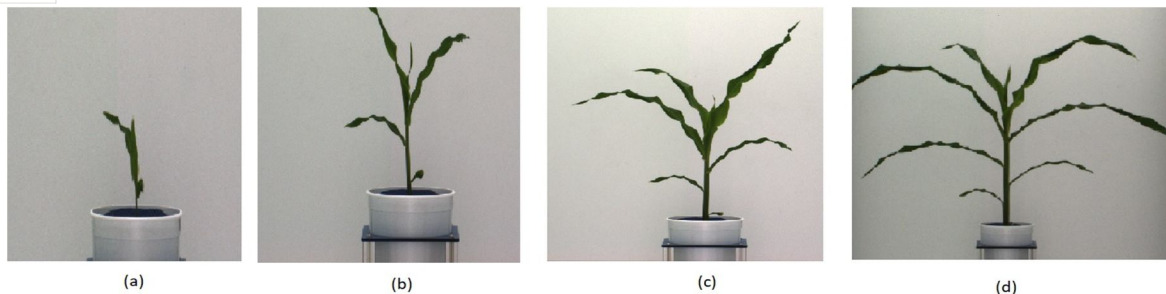


Fig.1. "Maturing Maize Plant: A Visual Journey from Seedling to Harvest"

#### IV. CHALLENGES

- 1) **Sample Size and Diversity:** The study might have a limited sample size or focus on specific crop varieties, which could restrict the generalizability of the results to other plant species or environmental conditions.
- 2) **Data Quality and Preprocessing:** The accuracy and reliability of high-throughput image analysis heavily depend on the quality of the acquired images and the effectiveness of preprocessing steps. Inaccuracies in segmentation or feature extraction could affect the validity of the conclusions.
- 3) **Experimental Design:** The paper might have potential issues related to experimental design, such as control treatments, randomization, or replication, that could impact the robustness of the conclusions.
- 4) **Measurement Precision:** Some phenotypic traits might be challenging to accurately measure using high-throughput imaging alone, leading to potential measurement errors or uncertainties.
- 5) **Environmental Variability:** The study may not fully account for the natural variability in environmental conditions, which could influence plant growth and drought responses.
- 6) **Data Analysis Methods:** The choice of data analysis methods and statistical approaches could influence the interpretation of results. Inappropriate or inadequate analysis methods might lead to biased or misleading conclusions.
- 7) **Incomplete Understanding of Plant Physiology:** While high-throughput imaging provides valuable insights, it might not capture all the underlying physiological mechanisms governing plant growth and drought responses.
- 8) **Publication Bias:** If the paper only reports significant results or positive outcomes, it may suffer from publication bias, potentially neglecting important negative findings.
- 9) **Data Availability:** The availability of the dataset used in the paper might not be fully open or accessible, limiting the reproducibility and transparency of the research.
- 10) **Time Frame:** As the paper was published in 2014, the research might not reflect the latest advancements in high-throughput image analysis or related technologies.

#### V. CONCLUSION

We conclude that using the Image processing techniques we easily analyze the plant phenotype. We learn about dataset and use various techniques for Extraction, Clustering and Classification. In this publication, we attempted to conduct a critical assessment of studies conducted by researchers in various parts of the world.

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