A low-cost greenhouse-based high-throughput phenotyping platform for genetic studies: A case study in maize under inoculation with plant growth-promoting bacteria

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Abstract
Greenhouse-based high-throughput phenotyping (HTP) presents a useful approach for studying novel plant growth-promoting bacteria (PGPB). Despite the potential of this approach to leverage genetic variability for breeding new maize (Zea Mays L.) cultivars exhibiting highly stable symbiosis with PGPB, greenhouse-based HTP platforms are not yet widely used because they are highly expensive; hence, it is challenging to perform HTP studies under a limited budget. In this study, we built a low-cost greenhouse-based HTP platform to collect growth-related image-derived phenotypes. We assessed 360 inbred maize lines with or without PGPB inoculation under nitrogen-limited conditions. Plant height, canopy coverage, and canopy volume obtained from photogrammetry were evaluated five times during early maize development. A plant biomass index was constructed as a function of plant height and canopy coverage. Inoculation with PGPB promoted plant growth in early developmental stages. Phenotypic correlations between the image-derived phenotypes and manual measurements were at least 0.47 in the later stages of plant development. The genomic heritability estimates of the image-derived phenotypes ranged from 0.23 to 0.54. Moderate-to-strong genomic correlations between the plant biomass index and shoot dry mass (0.24–0.47) and between HTP-based plant height and manually measured plant height (0.55–0.68) across the developmental stages showed the utility of our HTP platform. Collectively, our results demonstrate the usefulness of the low-cost HTP platform for large-scale genetic and management studies to capture plant growth.

Abbreviations: CC, canopy coverage; CV, canopy volume; GBLUP, genomic best linear unbiased prediction; GCP, ground control points; GDD, growing degree days; HTP, high-throughput phenotyping; LB, Luria-Bertani medium; NDVI, normalized difference vegetation index; NL, number of fully expanded leaves; PGPB, plant growth-promoting bacteria; PH, plant height; PH_{HTP}, high-throughput phenotyping plant height; RTK, real-time kinematic; SDM, shoot dry mass; SNP, single-nucleotide polymorphism; UAV, unoccupied aerial vehicle.

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1 | INTRODUCTION

Recent studies have reported the benefit of using plant growth-promoting bacteria (PGPB) to increase yield and resilience against biotic and abiotic stresses (Arif et al., 2020; Kumar et al., 2020) through various molecular mechanisms, including nitrogen fixation and phytohormone production (Compant et al., 2010; Manoj et al., 2020). Importantly, Wintermans et al. (2016) and Vidotti et al. (2019a) found a differential response of genotypes under PGPB inoculation, suggesting that the response has a genetic basis. These findings opened frontiers for a plant breeding program to breed new cultivars having highly stable PGPB responses (Vidotti et al., 2019b). However, the difficulty of monitoring a large number of lines across phenological growth stages under different inoculation conditions constrains our ability to analyze the genetics of dynamic PGPB responses.

With the advancement in genotyping technologies, phenotyping is considered a new bottleneck in plant breeding (Araus et al., 2018; Furban & Tester, 2011). Image-derived high-throughput phenotyping (HTP) presents a new avenue for automatic characterization of plants, owing to its capacity to generate difficult-to-measure phenotypes over time using advanced sensors and cameras (Araus & Cairns, 2014; Campbell et al., 2019; Mazis et al., 2020). Greenhouse-based HTP platforms have been developed to evaluate a number of plant responses, such as morphological (Brichet et al., 2017), disease (Thomas et al., 2018), and physiological (Wang et al., 2018) under microbial inoculants (Chai et al., 2021) and biotic and abiotic stresses (Araus & Cairns, 2014; Campbell et al., 2018). Therefore, leveraging HTP to evaluate hundreds or thousands of genotypes nondestructively under different management studies (Araus et al., 2018; Rouphael et al., 2018) is a promising approach to study the interaction between plant genotypes and PGPB. The choice of the HTP platform largely depends on the trade-off between the precision of phenotypes, the number of managements it can evaluate, and cost.

One major factor limiting the wide deployment of image-derived HTP in plant breeding programs is the high cost of setting up an HTP platform, especially for small breeding programs or research institutions. In field trials, an unoccupied aerial vehicle (UAV) is the commonly used cost-effective HTP technology to collect high-throughput data (Araus et al., 2018; Xie & Yang, 2020). In greenhouses, conveyor (plant-to-sensor) and benchtop (sensor-to-plant) type systems are often used for automated HTP platforms (Li et al., 2021). The conveyor type automatically transports potted plants into an imaging room. In contrast, the benchtop type is equipped with a computer-controlled mechanical arm that can automatically locate the position of a plant for phenotyping. Although both conveyor and benchtop systems support diverse cameras, their installation costs are expensive and may require modification of the existing greenhouse facilities. When there are budget constraints, researchers are motivated to build self-developed HTP platforms because large-scale greenhouse-based HTP platforms are produced mainly by commercial companies (Czedik-Eysenberg et al., 2018), which are forbiddingly expensive.

Several efforts have been made to develop a novel low-cost custom greenhouse HTP platform (Du et al., 2021; Zhou et al., 2018). The most common approach is to use a sliding track or cable railing system to move the imaging system that consists of the camera in the x and y axes. The images are processed using image stitching or photogrammetry techniques to obtain two- or three-dimensional phenotypes. However, this type of HTP platform is yet to be widely adopted in genetic and management studies because the number of genotypes or managements that can be accommodated is limited. Therefore, the objective of this study was to build a low-cost noncommercial sensor-to-plant greenhouse-based HTP platform using a multispectral camera that has the capacity to accommodate hundreds of maize (Zea Mays L.) lines and develop an image-processing pipeline to obtain growth-related image-derived phenotypes. We assessed the utility of the image-derived phenotypes by evaluating 360 genotypes under different PGPB management in the early stages of maize development.

2 | MATERIALS AND METHODS

2.1 | Low-cost high-throughput phenotyping platform

A low-cost greenhouse HTP platform was built, wherein the camera was positioned in a way that it obtained images from directly above the plants. The system was built in a conventional greenhouse with dimensions of $3.5 \times 11 \times 6$ m height, width, and length, respectively. A cooling wall and ventilation were used to maintain the desired temperature, and additional luminosity was supplied using LED lamps.

The image capture system was inspired by the UAV flight plans. It consists of two fixed parallel tracks (9 m) and one mobile perpendicular track (5 m). They were positioned 2.5 m above the ground. The two parallel tracks were fixed to
FIGURE 1. Summary of the image acquisition using a low-cost high-throughput phenotyping platform for greenhouse experiments. The dark and light blue lines indicate the y and x sliding tracks and support tracks, respectively. The green boxes indicate the positions of the electric motors. The blue square is the mobile platform that contains the multispectral camera and the sensor. Real time kinematic ground control points (RTK-GCP) were used to assemble the orthomosaics.

The greenhouse roof, as well as two support tracks to ensure stability and alignment. The parallel tracks move the perpendicular track along the x axis, whereas the perpendicular track moves the sensors along the y axis. Each track contained an individual 96-watt electric motor. These electric motors were controlled to achieve the desired overlap using a remote control system (Figure 1). The speed of the tracks was set at 0.16 m s\(^{-1}\). The two electric motors were synchronized to keep them aligned.

A medium-density fiberboard platform (20 × 20 cm) was designed to accommodate the multispectral camera, light sensor, and battery. The fiberboard platform was attached to the y axis mobile track. Four ground control points (GCP) geo-referenced with real-time kinematic (RTK) were used to assemble the orthomosaics. Top-view image data collection was performed between 12:00 and 13:00 with an overlap of 80% frontal and 70% lateral views. The multispectral camera used was a Parrot Sequoia (Parrot SA), including green (550 nm), red (660 nm), red-edge (735 nm), and near-infrared (790 nm).

2.2 Image processing and data extraction

Multispectral images were processed by assembling orthomosaics and the dense point cloud using Agisoft Metashape software (Agisoft LLC). The images were imported, aligned, and optimized using GCP. This was followed by the calculation of the dense point clouds and the stitching of orthomosaics.

The orthomosaics were analyzed using QGIS software (QGIS Development Team, 2021) to obtain a shapefile for each plot. The plots were manually identified, and a geometry point was assigned at the center of the plant. Then, a round positive buffer of 0.10 m was drawn for each plot. The shapefile of each plot was manually adjusted to reduce overlaps across plants. We applied image segmentation to the orthomosaics using the normalized difference vegetation index (NDVI) (Rouse et al., 1974) with a threshold of 0.35 to separate canopy vegetation from the background. The reflectance of each plot was calculated as the mean of each wavelength (green, red, red-edge, and near-infrared) using the R package FIELDimageR (Matias et al., 2020). The NDVI was calculated using the following formula: $\text{NDVI} = \frac{(\text{NIR} - \text{RED})}{(\text{NIR} + \text{RED})}$, where NIR and RED are the reflectances at the near-infrared and red wavelengths, respectively. Canopy coverage (CC) was calculated from the sum of the pixels in the canopy vegetation and transformed to cm\(^2\) based on the resolution of the orthomosaics (mm pixel\(^{-1}\)).

Dense cloud points were used to estimate plant height (PH\(_{HTP}\)) and canopy volume (CV). Each point from the dense cloud point was composed of GPS coordinates (latitude, longitude, and altitude in the universal transverse mercator). The dense cloud point data were processed using the R package lidR (Roussel & Auty, 2021). A round positive buffer of 0.01 m was generated at the center of each plant to obtain the corresponding points of each plot. PH\(_{HTP}\) was constructed from the difference between the 90 percentile of the top of the point cloud altitude and the pot altitude before plant germination (0 leaves) (Figure 2) (Galli et al., 2021). The image-derived plant biomass index, f(biomass), was derived from the product of PH\(_{HTP}\) (cm) and CC (cm\(^2\)) (Li et al., 2020) as $f(\text{biomass}) = \text{PH}_{HTP} \times \text{CC}$. For CV, the dense cloud points were filtered by colors using the “Select Points by Color” function in the Agisoft Metashape software to remove the background. Plants were then reconstructed from the point cloud data, and the CV was estimated using the $\alpha$-shape algorithm (Lafarge & Pateiro-Lopez, 2020). The algorithm requires an $\alpha$ value that controls the tightness of the three-dimensional reconstruction of the points. The...
optimal value of $\alpha$ that yielded the greatest correlation with manual measurements was 0.01 (Moreno et al., 2020).

2.3 Plant growth-promoting bacteria experiment

A tropical maize association panel containing 360 inbred lines was used to study the response to PGPB. Of these, 179 inbred lines were from the Luiz de Queiroz College of Agriculture-University of São Paulo (ESALQ-USP) and 181 were from the Instituto de Desenvolvimento Rural do Paraná.

The inbred lines were evaluated under two managements: with (B+) and without (B–) PGPB inoculation under nitrogen stress. The B+ management consisted of a synthetic population of four PGPB. Bacillus thuringiensis RZ2MS9, Delftia sp. RZ4MS18 (Batista et al., 2018; Batista et al., 2021), Pantoea agglomerans 33.1 (Quecine et al., 2012), and Azospirillum brasilense Ab-v5 (Hungria et al., 2010) were selected based on a preliminary experiment that showed their ability to promote growth when co-inoculated. Each species was grown individually in Luria-Bertani (LB) medium at 28 °C with agitation at 150 rpm for 24 h. The synthetic population was composed of an adjusted volume of each bacterial culture medium containing approximately $10^8$ colony-forming units/ml. The B– management consisted of an inoculum with liquid LB only. Each plot containing three seeds was individually inoculated with 1 ml of the respective management, agitated, and sown afterwards. Each line was replicated twice across time, and each replication was composed of an augmented block design with six blocks and three common checks. Each experimental unit consisted of one pot containing one plant. The managements B+ and B– were evaluated simultaneously.

A pot had 20 cm of diameter with 3 L of capacity. The pots were separated from each other at a distance of approximately 2 cm. To facilitate fertilization, irrigation, and weed removal, a space of approximately 50 cm for every six rows of pots was created. Detailed information about the experimental design and the PGPB inoculation is available in Yassue et al. (2021).

A total of 13,826 single-nucleotide polymorphisms (SNPs) were available for the maize inbred lines using a genotyping-by-sequencing method following the two-enzyme (PstI and MseI) protocol (Poland et al., 2012; Sim et al., 2012). DNA was extracted using the cetyltrimethylammonium bromide method (Doyle & Doyle, 1987). Single-nucleotide polymorphism calling was performed using the TASSEL 5.0.
software (Bradbury et al., 2007) with B73 (B73-RefGen_v4) as the reference genome. The SNP markers were filtered if the call rate was less than 90%, nonbiallelic, and the minor allele frequency was less than 5%. Missing marker codes were imputed using the Beagle 5.0 software (Browning et al., 2018). Markers with pairwise linkage disequilibrium higher than 0.99 were removed using the SNPRelate R package (Zheng et al., 2012).

2.4 Manually measured and high-throughput phenotypes

The experiments were performed at ESALQ-USP in Brazil (22°42′39″ S; 47°38′09″ W, altitude 540 m). The final evaluation was conducted when most plants had developed six fully expanded leaves, approximately 33 days after sowing. The growth-related manually measured traits that were evaluated were plant height (PH) and shoot dry mass (SDM). Plant height was measured from the soil to the last expanded leaf’s ligule, and SDM was obtained from the dry mass of the leaves and stalk.

The image-derived phenotypes were collected over time to capture plant growth, as previously described. For each replication, measurements were made at six time points defined by the number of expanded leaves: 0 (before germination), 2, 3, 4, 5, and 6 (Hanway, 1966). Since the genotypes presented expected inconsistencies in growth stages, the number of expanded leaves was determined as the mode of the population at a given time. A time point before the germination step was used to obtain the PH_{HTP}. Heat accumulation was calculated from the growing degree days (GDD) based on the formula: \( GDD = \sum_{i=1}^{m} (T_i - T_{base}) \), where \( T_i \) is the daily mean air temperature and \( T_{base} \) is the base temperature of 10 °C. Mean air temperature was calculated using the following formula: \( T_i = \frac{T_{max} + T_{min}}{2} \), where \( T_{max} \) and \( T_{min} \) are the maximum and minimum temperatures, respectively, of day \( i \) (Gilmore & Rogers, 1958). The R package pollen (Nowosad, 2019) was used to calculate GDD. Phenotypic correlations were estimated using Pearson correlations between the image-derived phenotypes (PH_{HTP}, CC, f(biomass), and CV) and manually measured phenotypes (PH and SDM).

2.5 Likelihood-ratio and Wald tests

The following model was used to test the effects of genotype, management (B+ and B−), and their interaction.

\[
y = 1\mu + X_1r + X_2b + X_3m + Z_1g + Z_2gm + \epsilon
\]

where \( y \) is the vector of phenotypes; \( 1 \) is the vector of ones; \( X_1, X_2, \) and \( X_3 \) are the incidence matrices for the fixed effects; \( Z_1 \) and \( Z_2 \) are the incidence matrices for the random effects; \( \mu \) is the overall mean; \( r, b, \) and \( m \) are the fixed effects for replication, block within replication, and management (B+ and B−), respectively; \( g \sim N(0, G\sigma^2_g) \) is the vector of random effect of genotype; \( gm \sim N(0, G \otimes I\sigma^2_{gm}) \) is the vector of random effects of the interaction between genotype and management; and \( \epsilon \sim N(0, \Sigma) \) is the random residual effect. Here \( G \) is the additive genomic relationship matrix (VanRaden, 2008); \( I \) is the identity matrix; \( \sigma^2_g \) is the additive genomic variance; \( \sigma^2_{gm} \) is the genotype × management interaction variance; and \( \sigma^2_\epsilon \) is the residual variance. The significance of random and fixed effects was assessed using the Wald and likelihood-ratio tests, respectively. The analysis was performed using the R package ASReml-R (Butler et al., 2017).

2.6 Conventional heritability

The conventional heritability (repeatability) was calculated for image-derived and manually measured phenotypes using the model described earlier, but the management (m) and genotype × management interaction terms (gm) were dropped. It was assumed that \( g \sim N(0, I\sigma^2_g) \) and \( \epsilon \sim N(0, \Sigma) \). The conventional heritability was estimated using the following formula:

\[
h^2 = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_\epsilon}{n_r}}
\]

where \( n_r \) is the number of replications (2).

2.7 Bayesian genomic best linear unbiased prediction

Univariate and bivariate Bayesian genomic best linear unbiased prediction (GBLUP) models were used to estimate genomic heritability and genomic correlation separately for B+ and B−. These Bayesian models were the same as those used for the Wald and likelihood-ratio tests, but the management (m) and genotype × management interaction terms (gm) were dropped. For the univariate model, a flat prior was assigned to \( r \) and \( b \). The variance components, \( \sigma^2_g \) and \( \sigma^2_\epsilon \), were drawn from a scaled inverse \( \chi^2 \) distribution. For the bivariate model, \( y \) is the vector of phenotypes of two responses; \( g \sim N(0, \Sigma_g \otimes G) \) is the vector of genotypes; \( \epsilon \sim N(0, \Sigma_\epsilon \otimes I) \) is the residual; \( \otimes \) is the Kronecker product; and \( \Sigma_g \) and \( \Sigma_\epsilon \) are the variance-covariance matrices for additive genomic and residual effects taking the forms of

\[
\Sigma_g = \begin{bmatrix} \sigma^2_{g1} & \sigma^2_{g12} \\ \sigma^2_{g21} & \sigma^2_{g2} \end{bmatrix}, \quad \Sigma_\epsilon = \begin{bmatrix} \sigma^2_{\epsilon1} & \sigma^2_{\epsilon12} \\ \sigma^2_{\epsilon21} & \sigma^2_{\epsilon2} \end{bmatrix}
\]
where subscripts 1 and 2 refer to the first and second phenotypes. An inverse Wishart distribution was assigned to $\Sigma_g$ and $\Sigma_\epsilon$ with degrees of freedom $\nu = 4$ and scale matrix $S$ such that the prior means of $\Sigma_g$ and $\Sigma_\epsilon$ equal half of the phenotypic variance. All the Bayesian GBLUP models were fitted using 60,000 Markov chain Monte Carlo samples, 10,000 burn-in, and a thinning rate of 60 implemented in JWAS software (Cheng et al., 2018a; Cheng et al., 2018b). Model convergence was assessed using trace plots of the posterior distributions of the variance components.

### 2.8 Genomic heritability and genomic correlation

The variance components obtained from the univariate Bayesian GBLUP were used to estimate genomic heritability using the following formula:

$$h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_\epsilon^2}$$

The estimates of genomic correlation were obtained from the estimated variance-covariance matrix in the bivariate Bayesian GBLUP model.

### 2.9 Data availability

The genotype and image data are available at https://doi.org/10.17632/5gvzd2b3n.3 and https://doi.org/10.17632/frsfpgnsyz.1, respectively.

### 3 RESULTS

#### 3.1 Image processing and data extraction

A total of 756 plots (plants) in each replication across time were evaluated during plant development. Each collection of images took approximately 10 min. The ground resolution of the orthomosaics was approximately 2.30 mm pix$^{-1}$, and the GCP error was approximately 4 cm (Table 1). Despite the difference between days after sowing, accumulated GDD were similar between Replication 1 and Replication 2. In addition, the ground resolution of the orthomosaic values and GCP errors were consistent across different numbers of leaves.

#### 3.2 Plant growth-promoting bacteria experiment

##### 3.2.1 Statistical hypothesis testing and phenotypic correlation

The summary statistics of manually measured and image-derived phenotypes are shown in Supplementary Figures S1 and S2. The management and genotype effects were statistically significant for all image-derived and manually measured phenotypes across the different stages of maize development (Supplementary Tables S1–S4). This suggests that the presence of PGPB and genetic diversity significantly affect plant development and growth for PH$_{HTP}$, CC, f(biomass), and CV. However, the genotype $\times$ management interaction was not statistically significant. Similarly, the main effects of management and genotype were consistently significant, but the genotype $\times$ management interaction was absent for manually measured PH and SDM (Supplementary Table S5). Figure 3 shows the growth patterns of the image-derived phenotypes with (B+) or without (B−) PGPB inoculation. The B+ management produced higher mean values than the B− management for all image-derived and manually measured phenotypes, suggesting that PGPB inoculation promotes plant growth in early developmental stages as expected. Moderate phenotypic correlations were observed between the HTP and manually measured phenotypes (Table 2 and Supplementary Figure S3). Phenotypic correlations between PH$_{HTP}$ and PH ranged from 0.23 to 0.64 (B+) and 0.36 to 0.57 (B−). Image-derived phenotypes CC, f(biomass), and CV were equally correlated with SDM. The later growth stages tended to show higher phenotypic correlations (four, five, and six leaves). Overall, B+ and B− showed a similar pattern of phenotypic correlations.

##### 3.2.2 Heritability

Estimates of conventional and genomic heritability varied across image-derived phenotypes and stages of maize growth development.

### Table 1 Replication (Rep), number of fully expanded leaves (NL), days after sowing (DAS), ground resolution of orthomosaic (GRO), ground control points (GCP) error, and accumulated growing degree days (GDD) across five evaluations during maize growth development.

| Rep | NL  | DAS | GRO     | GCP error | GDD* |
|-----|-----|-----|---------|-----------|------|
| 1   | 2   | 11  | 2.32    | 0.04      | 169.6|
| 1   | 3   | 15  | 2.29    | 0.06      | 229.3|
| 1   | 4   | 18  | 2.27    | 0.05      | 268.5|
| 1   | 5   | 22  | 2.25    | 0.05      | 320.4|
| 1   | 6   | 27  | 2.25    | 0.04      | 394.8|
| 2   | 2   | 14  | 2.34    | 0.03      | 172.1|
| 2   | 3   | 21  | 2.35    | 0.03      | 250.1|
| 2   | 4   | 27  | 2.31    | 0.03      | 310.6|
| 2   | 5   | 30  | 2.31    | 0.03      | 338.8|
| 2   | 6   | 37  | 2.29    | 0.03      | 410.3|

*The base temperature used for GDD estimation was 10 °C.
FIGURE 3  Growth patterns of genotypes across maize development with (B+) or without (B–) plant growth-promoting bacteria inoculation. The blue and red dashed lines represent the means of B+ and B– managements, respectively. Each thin colored line represents the mean of a genotype

TABLE 2  Phenotypic ($r_p$) and genomic ($r_g$) correlations between high-throughput phenotyping and manually measured phenotypes across maize development with (B+) or without (B–) plant growth-promoting bacteria inoculation

| PH$_{HTP}$:PH | CC:SDM | f(biomass):SDM | CV:SDM |
|---|---|---|---|
| $r_p$ | $r_g$ | $r_p$ | $r_g$ | $r_p$ | $r_g$ | $r_p$ | $r_g$ |
| B+ | | | | | | | |
| 2 | 0.29 | 0.55 | 0.35 | 0.20 | 0.35 | 0.24 | 0.42 | 0.14 |
| 3 | 0.23 | 0.59 | 0.45 | 0.30 | 0.39 | 0.39 | 0.62 | 0.29 |
| 4 | 0.61 | 0.64 | 0.51 | 0.35 | 0.62 | 0.47 | 0.38 | 0.36 |
| 5 | 0.64 | 0.67 | 0.47 | 0.30 | 0.64 | 0.43 | 0.62 | 0.32 |
| 6 | 0.54 | 0.66 | 0.53 | 0.32 | 0.60 | 0.42 | 0.65 | 0.31 |
| B– | | | | | | | |
| 2 | 0.38 | 0.60 | 0.35 | 0.35 | 0.38 | 0.41 | 0.29 | 0.21 |
| 3 | 0.36 | 0.67 | 0.51 | 0.41 | 0.48 | 0.46 | 0.49 | 0.29 |
| 4 | 0.53 | 0.68 | 0.59 | 0.47 | 0.59 | 0.43 | 0.40 | 0.36 |
| 5 | 0.57 | 0.63 | 0.62 | 0.45 | 0.66 | 0.47 | 0.50 | 0.32 |
| 6 | 0.56 | 0.67 | 0.63 | 0.32 | 0.63 | 0.44 | 0.61 | 0.31 |

Note. CC, canopy coverage; CV, canopy volume; f(biomass), plant biomass index; NL, number of fully expanded leaves; PH, manually measured plant height; PH$_{HTP}$, image-derived plant height; SDM, shoot dry mass.

development (Table 3 and Supplementary Table S6). Earlier developmental stages tended to show higher estimates of conventional and genomic heritability. Among image-derived phenotypes, PH$_{HTP}$ showed the highest estimates of genomic heritability ranging from 0.35 to 0.54 (B+) and 0.34 to 0.48 (B–). In contrast, CV showed the lowest genomic heritability estimates, particularly when the number of leaves was five. The conventional heritability estimates for manually measured PH were 0.59 (B+) and 0.57 (B–), while those of SDM were 0.16 for both managements. The genomic heritability estimates of manually measured PH were 0.61 (B+) and 0.57 (B–), while those of SDM were 0.30 (B+) and 0.28 (B–).
Overall, the difference in the heritability estimates between B+ and B– was small.

3.2.3 Genomic correlation

The genomic correlations between image-derived and manually measured phenotypes showed a similar tendency to those of phenotypic correlations (Table 2). High genomic correlations were observed between PH_{HTP} and PH in the later stages of maize development for both B+ and B–. The image-derived f(biomass) showed the strongest genomic correlations with SDM, followed by CC. No differences in genomic correlations were observed between B+ and B–. In addition, moderate-to-strong phenotypic and genomic correlations were observed across the developmental stages for each image-derived phenotype (Figure 4). As expected, measurements made at growth stages with adjacent number of leaves showed higher correlations.

4 DISCUSSION

A greenhouse HTP platform was developed to evaluate the influence of PGPB on plant growth using image-derived phenotypes. HTP platforms play an important role in plant breeding programs, genetics, and management studies because they allow the evaluation of plant growth and development in a non-destructive, time-efficient, and less laborious manner. The image-capture system and processing were designed to be similar to those of UAVs. The roof structure of the greenhouse was used to attach the tracks to save costs and enable easy installation without restructuring the greenhouse itself. The total cost to develop our greenhouse-based HTP system was approximately US$5,000. Our expenses were higher than those of a recently developed HTP system for soybean (Zhou et al., 2018). However, the size of the HTP platform developed in this study is larger and can accommodate more genotypes. In terms of cost per m², our HTP platform is still cost-efficient because the cost associated with our HTP system was $75 per m², whereas that of (Zhou et al., 2018) was $40 per m². Although the orthomosaics and the dense point clouds were constructed using the proprietary Agisoft Metashape software, one of the open-source alternatives is WebODM (Vacca, 2020).

The image overlap during the capture was controlled by the opening angle of the camera, speed of the track, and the y axis distance, so that different cameras can be easily utilized by adjusting these factors. The coordinate system used for GCP was a universal transverse mercator obtained from RTK GPS, which may not always work indoors because of the greenhouse roof. An alternative option is to use a local coordinate system.

The image analysis pipeline consisted of aligning the images, obtaining dense cloud points, and mosaicking (Figure 2). The most laborious steps were to manually identify each plot and adjust its shapefile to avoid overlapping plots. Several approaches have been proposed to automate the plot identification step, such as the fieldShape function in the FIELDimageR R package (Matias et al., 2020) or a negative buffer area (Galli et al., 2020). However, these methods did not produce adequate results in our case, probably because of leaf overlapping (Ahmed et al., 2019). An alternative emerging approach is to implement semantic segmentation and object detection based on deep learning (Xie et al., 2017; Zou et al., 2020).

The effects of genotype and management were significant and consistent between the image-derived and manually measured phenotypes. This suggests that image-derived phenotypes can be used to assess the differences within genotypes or managements. In addition, the image-derived phenotypes were capable of capturing plant growth at different stages of plant development. The image-derived genomic heritability estimates tended to be lower than those of manually measured phenotypes and decreased as the plants developed. This was likely due to the difficulty in accurately phenotyping taller plants. The magnitude of the genomic correlations and genomic heritabilities were similar between management groups B+ and B–. This was expected because the genotype × management interaction term was not significant. Our HTP platform was able to consistently capture genetic variability within each management.

No significant interaction between genotype and management for both HTP and manually measured phenotypes may also indicate the absence of phenotypic plasticity for PGPB responses in our population. Our findings agree with those of (Vidotti et al., 2019a) and (Vidotti et al., 2019b), who did not find significant genotype and management interactions in hybrid maize using different genotypes and PGPB from this study. This might be because both managements were tested under nitrogen-limited conditions, or the experiment only covered the early developmental stages. For example, Guo et al. (2020) reported that low nutrients in optimal

| NL | PH_{HTP} | CC | f(biomass) | CV |
|----|---------|----|------------|----|
| B+ | 0.54    | 0.48 | 0.46       | 0.46 |
| B– | 0.36    | 0.44 | 0.33       | 0.37 |
| B+ | 0.43    | 0.36 | 0.35       | 0.36 |
| B– | 0.41    | 0.44 | 0.23       | 0.22 |
| B+ | 0.35    | 0.34 | 0.23       | 0.24 |
| B– | 0.37    | 0.33 | 0.24       | 0.25 |

Note. CC, canopy coverage; CV, canopy volume; f(biomass), plant biomass index; NL, number of fully expanded leaves; PH, manually measured plant height; PH_{HTP}, image-derived plant height; SDM, shoot dry mass.

TABLE 3 Genomic heritability estimates of image-derived phenotypes across maize development with (B+) or without (B–) plant growth-promoting bacteria inoculation.
irrigated growth conditions might contribute to the absence of genotype × water availability interaction in wheat. On the other hand, the significant management effect suggests that PGPB can promote plant growth. Nevertheless, further studies are needed to vary nitrogen levels, assess PGPB responses at the later stages of development, and validate our results in field trials.

Moderate-to-strong phenotypic and genomic correlations between PH$_{HTP}$ and PH revealed that image-derived PH$_{HTP}$ can be a good predictor for manually measured PH. Similarly, a moderate genomic correlation between f(biomass) and SDM suggests that f(biomass) can be used as a secondary or correlated phenotype for SDM in genomic predictions (Rutkoski et al., 2016). We also investigated the utility of spectral indices (e.g., NDVI) as a proxy for SDM. However, the phenotypic correlation between NDVI and SDM was low (average was 0.13). A potential reason for this might be the difficulty in accurately calibrating images using a calibrated reflectance panel or a sunshine (light) sensor. The reduction of sunlight inside the greenhouse due to the polyethylene roof may have limited the calibration accuracy. Unlike Li et al. (2020), this was the main reason why we did not include NDVI to calculate f(biomass).

The architecture of maize plants makes image-derived phenotyping harder because stalks and leaves grow beyond their pots and interfere with neighboring pots. This can be minimized by increasing the distance between the pots and distributing them equidistantly if a larger greenhouse is available. Another limiting factor that may reduce the correlation between PH$_{HTP}$ and PH is related to plant morphology. For instance, during maize growth, the leaf development stage directly affects plant height projection. Alternatively, we can measure PH$_{HTP}$ at the leaf ligule of the last fully expanded leaf. However, locating the leaf ligule in the HTP platform is a challenging task because PH$_{HTP}$ is based on plant height projection (Figure S4).

There are several greenhouse-based HTP platforms available that differ in terms of precision, resolution, and applications (Li et al., 2021). The advantage of our HTP platform is its low cost compared with commercial platforms, while having the capacity to phenotype many lines. Despite the fact that our image-derived phenotypes were slightly less...
correlated with manually measured phenotypes than other related studies found in the literature (Campbell et al., 2015; Volpato et al., 2021; Zhou et al., 2018), our results confirm that image-derived phenotypes can provide valuable information for capturing temporal PGPB responses in maize. Further research is warranted to evaluate the utility of image-derived phenotypes to study PGPB responses in longitudinal genomic predictions and genome-wide association studies (Anderson et al., 2020; Baba et al., 2020; Campbell et al., 2019).

5 | CONCLUSIONS

We developed a low-cost high-throughput phenotyping platform capable of capturing plant growth across developmental stages. This platform was used to study the symbiosis between PGPB and maize. We found a moderate-to-strong phenotypic and genomic correlation between the image-derived and manually measured phenotypes, where PGPB promoted growth in early developmental stages in the population. The findings reported in this study will help small plant breeding programs or public research institutions to integrate phenomics, genetic, and management studies under a limited budget.

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AUTHOR CONTRIBUTIONS

Rafael Massahiro Yassue: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft; Writing – review & editing. Giovanni Galli: Investigation; Methodology; Writing – review & editing. Ronaldo Borsato Jr.: Investigation; Writing – review & editing. Hao Cheng: Software; Writing – review & editing. Gota Morota: Conceptualization; Methodology; Supervision; Writing – review & editing. Roberto Fritsche-Neto: Conceptualization; Funding acquisition; Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

Ahmed, I., Ermanian, M., Ovsyanickov, I., van der Kamp, W., Nielsen, K., Duddu, H. S., Rumali, A., Shiriliffe, S., & Bett, K. (2019). Automatic detection and segmentation of lentil crop breeding plots from multi-spectral images captured by UAV-mounted camera. In 2019 IEEE Winter Conference on Applications of Computer Vision (WACV) (pp. 1673–1681). IEEE.

Anderson, S. L., Murray, S. C., Chen, Y., Malambo, L., Chang, A., Popescu, S., Cope, D., & Jung, J. (2020). Unoccupied aerial system enabled functional modeling of maize height reveals dynamic expression of loci. Plant Direct, 4(5), e00223. https://doi.org/10.1002/pld3.223

Araus, J. L., & Cairns, J. E. (2014). Field high-throughput phenotyping: The new crop breeding frontier. Trends in Plant Science, 19(1), 52–61. https://doi.org/10.1016/j.tplants.2013.09.008

Araus, J. L., Kefauver, S. C., Zaman-Allah, M., Olsen, M. S., & Cairns, J. E. (2018). Translating high-throughput phenotyping into genetic gain. Trends in Plant Science, 23(5), 451–466. https://doi.org/10.1016/j.tplants.2018.02.001

Arif, I., Batool, M., & Schenk, P. M. (2020). Plant microbiome engineering: Expected benefits for improved crop growth and resilience. Trends in Biotechnology, 38(12), 1385–1396. https://doi.org/10.1016/j.tibtech.2020.04.015

Baba, T., Momen, M., Campbell, M. T., Walia, H., & Morota, G. (2020). Multi-trait random regression models increase genomic prediction accuracy for a temporal physiological trait derived from high-throughput phenotyping. Plos One, 15(2), e0228118. https://doi.org/10.1371/journal.pone.0228118

Batista, B. D., Dourado, M. N., Figueredo, E. F., Hortencio, R. O., Marques, J. P. R., Piotto, F. A., Bonatelli, M. L., Settles, M. L., Azevedo, J. L., & Quecine, M. C. (2021). The auxin-producing bacterium thuringiensis RZ2ms9 promotes the growth and modifies the root architecture of tomato (Solanum lycopersicum cv. Micro-Tom). Archives of Microbiology, 203, 3869–3882. https://doi.org/10.1007/s00203-021-02361-z

Batista, B. D., Lacava, P. T., Ferrari, A., Teixeira-Silva, N. S., Bonatelli, M. L., Tsui, S., Mondin, M., Kitajima, E. W., Pereira, J. O., Azevedo, J. L., & Quecine, M. C. (2018). Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. Microbiological Research, 206, 33–42. https://doi.org/10.1016/j.micres.2017.09.007

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics, 23(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Brichet, N., Fournier, C., Turc, O., Strauss, O., Artzet, S., Pradal, C., Welcker, C., Tardieu, F., & Cabrera-Bosquet, L. (2017). A robotic-assisted imaging pipeline for tracking the growths of maize ear and silks in a high-throughput phenotyping platform. Plant Methods, 13(1), 96. https://doi.org/10.1186/s13007-017-0246-7
Galli, G., Horne, D. W., Collins, S. D., Jung, J., Chang, A., Frutsche-Neto, R., & Rooney, W. L. (2020). Optimization of UAS-based high-throughput phenotyping to estimate plant health and grain yield in sorghum. The Plant Phenome Journal, 3(1), e20010. https://doi.org/10.1002/ppj2.20010

Galli, G., Sabadin, F., Costa-Neto, G. M. F., & Frutsche-Neto, R. (2021). A novel way to validate UAS-based high-throughput phenotyping protocols using in silico experiments for plant breeding purposes. Theoretical and Applied Genetics, 134(2), 715–730. https://doi.org/10.1007/s00122-020-03726-6

Gilmore, E. C., & Rogers, J. S. (1958). Heat units as a method of measuring maturity in corn I. Agronomy Journal, 50(10), 611–615. https://doi.org/10.3834/ajrngj1958.000219620050010

Guo, X., Svane, S. F., Füchtbauer, W. S., Andersen, J. R., Jensen, J., & Thorup-Kristensen, K. (2020). Genomic prediction of yield and root development in wheat under changing water availability. Plant Methods, 16(1), 90. https://doi.org/10.1186/s13007-020-00634-0

Hanway, J. J. (1966). How a corn plant develops. Iowa State University.

Hungria, M., Campo, R. J., Souza, E. M., & Pedrosa, F. O. (2010). Inoculation with selected strains of azospirillum brasilense and a. lipolyferum improves yields of maize and wheat in brazil. Plant and Soil, 331(1–2), 413–425. https://doi.org/10.1007/s11104-009-0262-0

Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S., & Verma, J. P. (2020). Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants. Frontiers in Microbiology, 11.

Lafarge, T., & Pateiro-Lopez, B. (2020). alphashape3d: Implementation of the 3D alpha-shape for the reconstruction of 3D Sets from a point cloud (R package version 1.3.1). https://cran.r-project.org/web/packages/alphashape3d/index.html

Li, D., Quan, C., Song, Z., Li, X., Yu, G., Li, C., & Muhammad, A. (2021). High-throughput plant phenotyping platform (HT3P) as a novel tool for estimating agronomic traits from the lab to the field. Frontiers in Bioengineering and Biotechnology, 8, 1533. https://doi.org/10.3389/fbioe.2020.623705

Li, F., Piasecki, C., Millwood, R. J., Wolfe, B., Mazarei, M., & Stewart Jr, C. N. (2020). High-throughput switchgrass phenotyping and biomass modeling by uav. Frontiers in Plant Science, 11, 1532. https://doi.org/10.3389/fpls.2020.57407

Manoj, S. R., Karthik, C., Kadirvelu, K., Arulsevi, P. I., Shanmugasundaram, T., Bruno, B., & Rajkumar, M. (2020). Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. Journal of Environmental Management, 254, 109779. https://doi.org/10.1016/j.jenvman.2019.109779

Matias, F. I., Caraza-Harter, M. V., & Endelman, J. B. (2020). FIELDimageR: An R package to analyze orthomosaic images from agricultural field trials. The Plant Phenome Journal, 3(1), e20005. https://doi.org/10.1002/ppj2.20005

Mazis, A., Choudhury, S. D., Morgan, P. B., Stoeger, V., HUller, J., Ge, Y., & Awada, T. (2020). Application of high-throughput plant phenotyping for assessing biophysical traits and drought response in two oak species under controlled environment. Forest Ecology and Management, 465, 118101. https://doi.org/10.1016/j.foreco.2020.118101

Moreno, H., Rueda-Ayala, V., Ribeiro, A., Bengoecha-Guevara, J., Lopez, J., Petteinatos, G., Valero, C., & Andújar, D. (2020). Evaluation of vineyard cropping systems using on-board RGB-depth perception. Sensors, 20(23), 6912. https://doi.org/10.3390/s20236912

Nowosad, J. (2019). pollen: Analysis of aerobiological data (R package version 0.71). https://cran.r-project.org/web/packages/pollen

Poland, J. A., Brown, P. J., Sorrells, M. E., & Jannink, J.-L. (2012). Development of high-density genetic maps for barley and wheat using...
a novel two-enzyme genotyping-by-sequencing approach. *Plos One*, 7(2), e32253. https://doi.org/10.1371/journal.pone.0032253

QGIS Development Team. (2021). *QGIS Geographic Information System*. QGIS Association.

Quecine, M. C., Araújo, W. L., Rossetto, P. B., Ferreira, A., Tsui, S., Lacava, P. T., Mondon, M., Azevedo, J. L., & Pizzirani-Kleiner, A. A. (2012). Sugarcane growth promotion by the endophytic bacterium pantoea agglomerans 33.1. *Applied and Environmental Microbiology*, 78(21), 7511–7518. https://doi.org/10.1128/AEM.00836-12

Rouphael, Y., Spichal, L., Panzarová, K., Casa, R., & Colla, G. (2018). High-throughput plant phenotyping for developing novel biostimulants: From lab to field or from field to lab? *Frontiers in Plant Science*, 9.

Rouse, J. W., Haas, R. H., Schell, J. A., & Deering, D. W. (1974). Monitoring vegetation systems in the great plains with ERTS. In S. C. Freiden, E. P. Mercanti, & M. A. Becker (Eds.) *Third Earth Resources Technology Satellite-1 Symposium: Vol. I. Technical presentations* (NASA SP-351) (p. 309). NASA.

Roussel, J.-R., & Auty, D. (2021). Airborne LiDAR data manipulation and visualization for forestry applications (R package version 3.1.4). https://cran.r-project.org/web/packages/lidR/index.html

Rutkoski, J., Poland, J., Mondal, S., Autrique, E., Pérez, L. G., Crossa, J., Reynolds, M., & Singh, R. (2016). Canopy temperature and vegetation indices from high-throughput phenotyping improve accuracy of pedigree and genomic selection for grain yield in wheat. *G3: Genes|Genomes|Genetics*, 6(9), 2799–2808. https://doi.org/10.1534/g3.116.032888

Sim, S.-C., Durstewitz, G., Plieske, J., Wieseke, R., Ganal, M. W., Deynze, A. V., Hamilton, J. P., Buell, C. R., Causse, M., Wijeratne, S., & Francis, D. M. (2012). Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. *Plos One*, 7(7), e40563. https://doi.org/10.1371/journal.pone.0040563

Thomas, S., Behmann, J., Steier, A., Kraska, T., Muller, O., Rascher, U., & Mahlein, A.-K. (2018). Quantitative assessment of disease severity and rating of barley cultivars based on hyperspectral imaging in a non-invasive, automated phenotyping platform. *Plant Methods*, 14(1), 45. https://doi.org/10.1186/s13007-018-0313-8

Vacca, G. (2020). WEB open drone map (WebODM): A software open source to photogrammetry process. In *Fig Working Week 2020: Smart surveyors for land and water management, May 10–14*, Amsterdam, the Netherlands.

VanRaden, P. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, 91(11), 4414–4423. https://doi.org/10.3168/jds.2007-0980

Vidotti, M. S., Lyra, D. H., Morosini, J. S., Granato, Í. S. C., Quecine, M. C., de Azevedo, J. L., & Fritsche-Neto, R. (2019a). Additive and heterozygous (dis)advantage GWAS models reveal candidate genes involved in the genotypic variation of maize hybrids to Azospirillum brasilense. *Plos One*, 14(9), e0222788. https://doi.org/10.1371/journal.pone.0222788

Vidotti, M. S., Matias, F. I., Alves, F. C., Pérez-Rodriguez, P., Beltran, G. A., Burgueño, J., Crossa, J., & Fritsche-Neto, R. (2019b). Maize responsiveness to Azospirillum brasilense: Insights into genetic control, heterosis and genomic prediction. *Plos One*, 14(6), e0217571. https://doi.org/10.1371/journal.pone.0217571

Volpato, L., Pinto, F., González-Pérez, L., Thompson, I. G., Borém, A., Reynolds, M., Gérard, B., Molero, G., & Rodrigues, F. A. (2021). High throughput field phenotyping for plant height using UAV-based RGB imagery in wheat breeding lines: Feasibility and validation. *Frontiers in Plant Science*, 12.

Wang, H., Qian, X., Zhang, L., Xu, S., Li, H., Xia, X., Dai, L., Xu, L., Yu, J., & Liu, X. (2018). A method of high throughput monitoring crop physiology using chlorophyll fluorescence and multispectral imaging. *Frontiers in Plant Science*, 9.

Winternam, P. C. A., Bakker, P. A. H. M., & Pieterse, C. M. J. (2016). Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. *Plant Molecular Biology*, 90(6), 623–634. https://doi.org/10.1007/s11103-016-0442-2

Xie, C., Wang, J., Zhang, Z., Zhou, Y., Xie, L., & Yuille, A. (2017). Adversarial examples for semantic segmentation and object detection. In *Proceedings of the IEEE international conference on computer vision* (pp. 1369–1378). IEEE.

Xie, C., & Yang, C. (2020). A review on plant high-throughput phenotyping traits using UAV-based sensors. *Computers and Electronics in Agriculture*, 178, 105731. https://doi.org/10.1016/j.compag.2020.105731

Yassue, R. M., Carvalho, H. F., Gervatsky, R., Sabadin, F., Souza, P. H., Bonatelli, M. L., Azevedo, J. L., Quecine, M. C., & Fritsche-Neto, R. (2021). On the genetic architecture in a public tropical maize panel of the symbiosis between corn and plant growth-promoting bacteria aiming to improve plant resilience. *Molecular Breeding*, 41(10). https://doi.org/10.1007/s11032-021-01257-6

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, 28(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

Zhou, J., Chen, H., Zhou, J., Fu, X., Ye, H., & Nguyen, H. T. (2018). Development of an automated phenotyping platform for quantifying soybean dynamic responses to salinity stress in greenhouse environment. *Computers and Electronics in Agriculture*, 151, 319–330. https://doi.org/10.1016/j.compag.2018.06.016

Zou, H., Lu, H., Li, Y., Liu, L., & Cao, Z. (2020). Maize tassels detection: A benchmark of the state of the art. *Plant Methods*, 16(1), 108. https://doi.org/10.1186/s13007-020-00651-z

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