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

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ABSTRACT

Exploring the symbiosis between plants and plant-growth-promoting bacteria (PGPB) is a new challenge for sustainable agriculture. Even though many works have reported the beneficial effects of PGPB in increasing plant resilience for several stresses, its potential is not yet widely explored. One of the many reasons is the differential symbiosis performance depending on the host genotype. This opens doors to plant breeding programs to explore the genetic variability and develop new cultivars with higher responses to PGPB interaction and, therefore, have higher resilience to stress. Hence, we aimed to study the genetic architecture of the symbiosis between PGPB and tropical maize germplasm, using a public association panel and its impact on plant resilience. Our findings reveal that the synthetic PGPB population can modulate and impact root architecture traits, improve resilience to nitrogen stress, and 37 regions were significant for controlling the symbiosis between PGPB and tropical maize. In addition, we found two overlapping SNPs in the GWAS analysis indicating strong candidates for further investigations. Furthermore, genomic prediction analysis with genomic relationship matrix computed using only significant SNPs obtained from GWAS analysis substantially increased the predictive ability for several traits endorsing the importance of these genomic regions for the response of PGPB. Finally, the public tropical panel reveals a significant genetic variability to the symbiosis with the PGPB and can be a source of alleles to improve plant resilience.

KEYWORDS

Shovelomics; root architecture; GWAS; symbiosis interaction; genomic prediction

DECLARATIONS

Funding and acknowledgment

This study was financed in part by São Paulo Research Foundation (FAPESP, Process: 19/04697-2; 17/24327-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

Genomic data: https://data.mendeley.com/datasets/5gvznd2b3n

Code availability

Code for image processing: https://github.com/RafaelYassue/Root-phenotyping

Ethics approval

Not applicable because this article does not contain any studies with human or animal subjects.

Consent to participate

Not applicable because this article does not contain any studies with human or animal subjects.

Consent for publication

All authors have approved the manuscript and agreed with submission to the Molecular Breeding journal.

INTRODUCTION

Due to the growing need for food (Ray et al. 2013) and environmental pressure (Qi et al. 2018), new approaches that increase production in a sustainable way are required (Gaffney et al. 2019). Tropical agriculture will have to rise to meet the food demand in tropical developing nations (Laurance et al. 2014). Also, studies estimate that it will be necessary to increase the use of fertilizers, with emphasis on N, P_20_5, and K_2O (Pradhan et al. 2015). Furthermore, multidisciplinary research that aims to increase production, sustainability, and plant resilience is justified.

The use of plant growth-promoting bacteria (PGPB) has been a promising field of interest due to the ability to increase production (Martins et al. 2018) and the resilience of the host caused by direct and indirect mechanisms. The most common mechanism is related to biofertilization, which consists of nutrient uptake and hormones production as well as the ones related to the improvement of plant defense (reviewed by (Santoyo et al. 2016; Vejan et al. 2016)). Many studies have proved the benefits of PGPB reducing the plant abiotic stresses caused by salinity (Rojas-Tapias et al. 2012), heavy metals (Gamalero et al. 2009), and drought (Sandhya et al. 2010). However, the use of PGPB as inoculants in agriculture is still incipient.

One of the main challenges of an inoculant with PGPB is the reproducibility of its results in the field (Bashan et al. 2014). Studies have shown that PGPB is influenced by several factors, such as soil type (Egamberdiyeva 2007), nitrogen fertilization (Rodríguez-Blanco et al. 2015), microbe-microbe interaction (Gaiero et al. 2013), and plant-PGPB interactions (Wintermans et al. 2016). In this sense, during the development of a new PGPB inoculant, both environmental and genetic factors must be considered (Lemanceau et al. 2017).

In recent years, the genotyping cost has decreased substantially, and its benefits have become consolidated. Currently, one of the gaps for greater gains using genotyping is the poor ability to phenotyping. High-throughput phenotyping (HTP) is a suitable alternative to increasing phenotyping power (Araus and Cairns 2014). One of the benefits of HTP is measuring secondary traits with direct or indirect effects on primary characteristics (Qiao et al. 2019). For roots, shovelomic analysis has been used to study the root system architecture traits and their impact on the final phenotype (Trachsel et al. 2011).

Root architecture is responsible for the nutrients and water uptake and its evaluations and selection have been the focus of plant breeding for many years (York et al. 2015). In maize, root traits have been identified as key phenotypes to overcome stress in specific environments (Mi et al. 2010; Lynch 2013; Adebayo et al. 2020). Also, in PGPB studies, the root and its architecture play a significant role in the direct interaction with the soil microbiome (Compant et al. 2010). Studies have also revealed that PGPB can modulate root architecture (Gutiérrez-Luna et al. 2010), and the host genotype can influence this response (Wintermans et al. 2016).

Modern plant breeding may have caused a bottleneck in genetic diversity for the symbiosis with PGPB due to the lower response in modern varieties (Valente et al. 2020). In maize, landraces reveal the ability to fix 29%-82% of the plant nitrogen through interaction with PGPB (Van Deynze et al. 2018). Additive and dominance effects have been reported (Vidotti et al. 2019b; Wagner et al. 2020), providing insights into the importance of the host genotype in the impact of PGPB response. GWAS studies have been employed to discover candidate genes to the response of PGPB in *Arabidopsis* (Wintermans et al. 2016; Proietti et al. 2018; Cotta et al. 2020) and maize (Vidotti et al. 2019a).

Based on the genetic variability for the response of PGPB inoculation in many crops, the response of maize to the symbiosis of PGPB may be improved with the support of plant breeding (Kroll et al. 2017; Wei and Jousset 2017) and contribute to more sustainable food production through the increase of yield, sustainability, and plant resilience. Therefore, we aimed to study the symbiosis's genetic architecture between the tropical maize germplasm and a synthetic population of plant growth-promoting bacteria. Also, we present a pipeline for shovelomics evaluation and analysis. Hence, this information may contribute to plant breeding programs, focusing on new strategies to produce more resilient crops.

MATERIALS AND METHODS

Public tropical maize panel

Our tropical maize germplasm panel contains 360 inbred lines used to analyze the symbiosis's genetic architecture between maize and PGPB. Among them, 179 inbred lines are from ESALQ-USP

(Luiz de Queiroz College of Agriculture-University of Sao Paulo) and 181 from IAPAR (Instituto de Desenvolvimento Rural do Paraná). The genomic and phenotypic information about this panel is available on the Mendeley platform (https://data.mendeley.com/datasets/5gvznd2b3n).

Bacterial strain and inoculum

The PGPB *Bacillus thuringiensis* RZ2MS9 and *Delftia* sp. RZ4MS18 was isolated from *Paullinia cupana* (Batista et al. 2018, Batista et al. 2021), *Pantoea agglomerans* 33.1 was isolated from *Eucalyptus grandis* (Quecine et al. 2012), and *Azospirillum brasilense* Ab-v5 is a commercial inoculant (Hungria et al. 2010). They were selected based on previous studies that have reported them as potential inoculants (Batista et al. 2018, Quecine et al. 2012, Hungria et al. 2010) to compose the synthetic population. *In vivo* trials revealed that these PGPB did not have antagonistic effects among each other and when co-inoculated, promoted growth in maize (unpublished data).

The synthetic population inoculum was prepared by growing each bacterium individually in Luria-Bertani (LB) medium at 28°C with 150 rpm agitation for 24h. The concentration of each bacterium was measured in a spectrophotometer. The synthetic population was composed of each bacteria's adjusted culture medium containing approximately 10^8 colony-forming units/mL. The treatment without PGPB consisted of preparing the inoculum with liquid LB only. Each plot containing three seeds was individually inoculated with 1 ml of the respective treatments, agitated, and sown afterward.

Greenhouse experiment

The experiments were carried out under greenhouse conditions at Luiz de Queiroz College of Agriculture (ESALQ/USP), Brazil (22˚42'39 "S; 47˚38'09 "W, altitude 540 m). The 360 inbred lines were evaluated in two experiments: with (B+) and without (B-) PGPB inoculation. Each experiment was conducted in an augmented blocks design with two replicates across time, each one consisting of six blocks with 60 inbred lines and three common checks. Each replicate of B+ and B- experiments were installed together in a greenhouse. The treatments B+ and B- were physically separated due to the ability of the PGPB to migrate from one to another (Chi et al. 2005; Ji et al. 2010). Furthermore, we calculated

the difference between the treatments B+ and B- to compound the Delta. Lastly, we performed analyzes considering the values from B+ and B- individually and the Delta value as a response to the inoculation.

In order to evaluate the resilience to N stress and identify genomic regions responsible for the symbiosis between PGPB and maize, we tested the genotypes with and without inoculation with PGPB (B+ and B-, respectively) in low nitrogen conditions similar to (Vidotti et al. 2019a). The low nitrogen condition consisted of no external nitrogen input, and all the nitrogen available to the plants was due to the natural soil organic matter or fixed from PGPB.

The maize plants were grown in 3-L plastic pots containing soil. Chemical and physical soil analysis is available in supplementary table 1. The planting fertilization was done according to soil nutrient content and crop demands provided by Soil-app (Matias et al. 2021). It consisted of potassium chloride, simple superphosphate, and limestone soil conditioner inputs added and mixed into the soil. Each plot was sown with three seeds, and after germination, the seedlings were thinning to only one. During the experiments, temperature, radiation, and humidity were monitored and are available in the supplemental figure 1. Twice a week, 200 ml of a complementary fertilizer without nitrogen and adapted from Hoagland and Snyder (1933) were applied in each plot. Irrigation and other cultural practices were carried out according to the needs of the crop.

Evaluations began when most of the plants were in the V6 growth stage (six expanded leaves). Plant height (PH, cm) was measured from the soil to the last expanded leaf's collar, and the number of expanded leaves was counted (NL). Afterward, the plants were cropped at the soil base, and the stalk diameter was measured using a digital caliper (SD, mm). Finally, the harvested shoot (leaves and stalk) was dried in a forced draft oven at 60°C for 72h to obtain shoot dry mass (SDM, g).

The roots were carefully washed with water, and each root was stored in plastic pots with a 25% ethanol solution for preservation. First, root images were taken to calculate root angle (RA, degree, °) and convex hull area (CHA, cm^2) using a Nikon CoolPix S8100 camera attached to a platform with a fixed height and position. For RA, the images were cropped in order to consider the first 10 centimeters representing the topsoil. These images were processed using a Python script that is available on GitHub (https://github.com/RafaelYassue/Root-phenotyping). Then, new root images were acquired by an Epson LA2400 scanner and processed using the WinRHIZO (Reagent Instruments Inc., Quebec, Canada) to

obtain lateral and axial root volume (LRV, ARV, cm^3, respectively), root length (RL, cm), and root average diameter (RAD, mm). Representative images of root phenotypes are available in Supplementary figure 5. Next, the roots were dried out to determine the root dry mass (RDM, g). Furthermore, the ratio of shoot/root (RSR, g g^{-1}) was obtained by dividing the SDM by the RDM.

Phenotypic analysis

To test the interaction between genotype and treatment (B+ and B-) we used the followed full model:

$$\mathbf{y} = \mathbf{X}_1\mathbf{t} + \mathbf{X}_2\mathbf{NL} + \mathbf{Z}_1\mathbf{r} + \mathbf{Z}_2\mathbf{b} + \mathbf{Z}_3\mathbf{g} + \mathbf{Z}_4\mathbf{gt} + \varepsilon \qquad \text{Eq. 1}$$

where **y** refers to the phenotypic observation vector, $\mathbf{X}_1$ and $\mathbf{X}_2$ are the incidence matrix for the fixed effect, $\mathbf{Z}_1$, $\mathbf{Z}_2$,$\mathbf{Z}_3$, and $\mathbf{Z}_4$ are the incidence matrices for the random effects. **t** is the fixed effects of treatment (B+ and B-); **r** is random effects of replicates, where $\mathbf{r} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_r)$; **b** is random effects of the block within replicates, where $\mathbf{b} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_b)$; **g** is the vector of random effects of genotype values, where $\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_g)$; **gt** is the vector of random effects of the interaction between genotype and treatment, where $\mathbf{gt} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_{gt})$; and ε is the random residual effects, where $\varepsilon \sim N(\mathbf{0}, \mathbf{I}\sigma^2_e)$. The random effects were tested using the likelihood ratio test (LRT) and the fixed effects using the Wald test. In order to correct the germination and seed vigor differences, the number of leaves (**NL**) was used as a covariable. Spatial analysis and unstructured residual effects were tested, and a good fit of the model was not reached.

After, we obtained the entry-mean heritabilities for each combination of trait and treatment using the reduced model for each treatment (B+,B-, and Delta):

$$\mathbf{y} = \mathbf{X}_1\mathbf{NL} + \mathbf{Z}_1\mathbf{g} + \mathbf{Z}_2\mathbf{r} + \mathbf{Z}_3\mathbf{b} + \varepsilon \qquad \text{Eq. 2}$$

The heritabilities using the full and reduced model (B+, B-, Delta) were calculated at the entry-mean level with the variance components from Eq. 1 and Eq. 2 using the following equations, respectively:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gt}^2}{\mathrm{t}} + \frac{\sigma_{\mathrm{t}}^2}{\mathrm{t\,r}}} \qquad \text{Eq. 3}$$

$$\mathrm{h}^2 = \frac{\sigma_{\mathrm{g}}^2}{\sigma_{\mathrm{g}}^2 + \frac{\sigma_{\mathrm{e}}^2}{\mathrm{r}}} \qquad \text{Eq. 4}$$

In which h^2 refers to the mean-entry heritability,σ^2_g, σ^2_{gt}, and σ^2_e are the variance components due to genotype, the interaction between genotype x treatment, and residual effects, respectively, and r and t is the number of replicates (r = 2) and treatment (t = 2), respectively. The analysis was performed using ASReml-R 4.0 (Butler et al. 2017).

Principal component analysis and Pearson correlation were performed to understand the association between traits. The interaction plot was performed using the R package Raincloud plots (Allen et al. 2021). The Levene's test was used to assess the equality of variances for treatment effects. To simplify a three-way interaction for genotype x treatment x traits, we used a reduced model considering treatment effects as a standardized mean of each treatment (B+, B-, Delta) for each trait. Then, a treatment by trait biplot (TT) was used to study the associations of traits across treatment. A two-way table containing the mean of each trait in each treatment was used to obtain the singular value decomposition and the biplot using the R package metan (Olivoto and Lúcio 2020). TT biplot analysis was adapted from genotype by trait biplot, and a detailed explanation about the analysis can be found in Yan et al (2018).

Genotypic data

All 360 tropical inbred lines were genotyped using a genotyping-by-sequencing (GBS) method following the two enzymes (*Pst*I and *Mse*I) protocol (Sim et al. 2012; Poland et al. 2012). For that, the DNA of tropical maize lines was extracted from young and healthy leaves using the CTAB protocol (Doyle and Doyle 1987). Individual genomic DNA samples were digested by restriction enzymes, and samples were included in a sequencing plate. The sequencing was performed on the Illumina NextSeq 500 platform (Illumina Inc., San Diego, CA, United States). Sequence data were aligned against the B73

(B73-RefGen_v4) maize reference genome, and the SNP calling was performed using the software TASSEL 5.0 (Bradbury et al. 2007) under default parameters values. The SNP dataset was filtered, and markers with call rate < 90%, non-biallelic, minor allele frequency (MAF) lower than 5%, and heterozygous loci on at least one individual were removed from the dataset, and remaining missing data were imputed by Beagle 5.0 algorithm (Browning et al. 2018). In addition, markers with pairwise linkage disequilibrium (LD) higher than $r^2 > 0.99$ were removed using the SNPRelate package (Zheng et al. 2012). Finally, a total of 13,826 SNPs were considered for the genomic analyses. The coverage and depth of the genotyping-by-sequencing data are shown in supplementary figure 6.

Population structure and LD decay

The principal components analysis (PCA) regarding the population structure was calculated based on the additive genomic relationship matrix (VanRaden 2008). To identify the most likely number of groups (K) in our panel, we estimated the optimal K-value based on the inferred number of groups producing the lowest cross-validation error using the software ADMIXTURE (Alexander and Lange 2011). We ran the software assuming 2–50 subpopulations using default parameters. We estimated the LD (r^2) between all SNP within a distance lower than 1 Mbp in the same chromosome, and r^2 values were plotted against base-pair distance to obtain the LD decay by chromosome. A second-degree locally weighted scatterplot smoothing (LOESS) function was used to draw a trend line for detecting the LD decay with a threshold of 0.1 (Esteras et al. 2013, Zhu et al. 2008).

GWAS analyses

The GWAS analyses were carried out for each combination of trait and inoculation (B+ and B-) and the difference between B+ and B- (Delta) using the FarmCPU R package (Liu et al. 2016). We tested models containing 0 to 6 principal components to correct the population structure effect, and the best model fit was based on QQplot. The Manhattan and QQ plots were obtained using the CMplot R package (Yin et al. 2020). We used the *FarmCPU.P.Threshold* function with 100 permutations to obtain

a *p* threshold for each trait. The significant SNPs were annotated using a windows range upstream and downstream, based on the LD decay of respective chromosomes (Supplementary figure 8). The MaizeMine V1.3. was used to obtain the genes on that window (Shamimuzzaman et al. 2020).

Genomic prediction

Genomic predictions (GP) analyses were conducted for each combination of trait x treatment (B+, B-, and Delta). Due to the non-significant effects of the interaction, we did not consider interaction models for the GP analysis. For GP, we used the following three models:

$$\hat{\mathbf{g}} = 1u + \mathbf{Za} + \varepsilon \qquad \text{GBLUP}$$

$$\hat{\mathbf{g}} = 1u + \mathbf{Za} + \mathbf{SNPs} + \varepsilon \qquad \text{GBLUP\_MAS}$$

$$\hat{\mathbf{g}} = 1u + \mathbf{Za} + \varepsilon \qquad \text{GBLUP\_GMS}$$

where $\hat{\mathbf{g}}$ is the vector of the adjusted mean for genotype for each treatment (B+, B-, and Delta) from Eq. 2, considering genotype as a fixed effect. **Z** is the incidence matrix of random effects of genotypes, and **a** is the vector of additive effects, where $\mathbf{a} \sim N(0, \mathbf{G}\,\sigma^2_a)$. ε is the vector of random residuals with $\varepsilon \sim N(0, \mathbf{I}\,\sigma^2_\varepsilon)$. **G** is the additive genomic relationship matrix (VanRaden 2008).

For the GBLUP_MAS model, SNPs are the fixed effects of the significant SNPs obtained from the GWAS analysis for each treatment (B+ and B-), except for Delta that the matrix was composed of all significant SNPs from B+, B-, and Delta GWAS analysis. For the model GBLUP_GMS (GWAS Marker Selection GBLUP), genomic additive matrix (**G**) was obtained using only the significant SNPs from the GWAS analysis for each treatment (B+ and B-), except for Delta that we used all significant SNPs. A similar approach for marker selection based on GWAS for genomic prediction is described by Jeong et al. (2020). The covered of the significant SNPs from GWAS analysis of each treatment (B+, B-, and Delta) were obtained using a windows range upstream and downstream, based on LD decay of respective chromosomes.

In order to evaluate the model performance, we used the CV-α cross-validation with 5 folds and 4 replications (Yassue et al. 2021). The predictive ability of each model was calculated by the Pearson correlation between the predicted and observed values from the validation set.

RESULTS

Exploratory analysis, significance, and heritability

The phenotypic data revealed that the synthetic population of PGPB did promote growth in maize for most traits (Figure 1 A). For SDM and RDM, the inoculation with PGPB promoted an increase of 12.78% and 20.65%, respectively. Regarding the root's architecture traits, they were also influenced by the inoculation. The plants inoculated with PGPB (B+) tended to have a higher mean for most root traits, except RA and RSR. Conversely, for RA, RAD, and RSR, the treatment without PGPB (B-) tended to have a higher phenotypic variation. TT biplot analysis revealed the association between traits and treatments. RAD, RDSM, RSR, RA, and SDM showed higher variance than other traits. On the other hand, PH, CHA, SD, and RL had smaller discrepancies across treatments. RDM and SD were associated with the Delta treatment, while SDM was associated with B+ (Figure 1 B.).

The traits most correlated with SDM were RDM (0.80), ARV (0.71), RL (0.64), LRV (0.56), SD (0.58), PH (0.50), and CHA (0.40). The root traits were also highly correlated (RDM x RL, LRV x RL, LRV x RDM, ARV x RDM). Overall, the correlation between traits for the treatment B+ and B- were similar. Although, for RAD x CHA, RDM x PH, SD x RAD, SDM x RAD, treatment B- had higher values, except for RL x CHA (Supplementary Figure 3). According to PCA analysis (Supplementary Figure 4), we observed that most of the traits were positively associated with bacteria inoculation, except for RA and RSR. Also, it was possible to cluster the genotypes by treatment (B+ and B-).

Significant effects for treatment and genotypes were observed for most traits. On the other hand, the interaction between genotype and treatment was not observed (Supplementary Table 2). Nevertheless, Levene's test reveals a difference between the variances in the treatment B+ and B- for all traits except for CHA, SDM, and PH. Furthermore, the heritabilities varied between treatment and among traits. PH and SD had the higher heritabilities, meanwhile, root-traits and Delta treatment heritabilities tended to be lower (Table 1).

Figure 1. A. Interaction plot considering 360 genotypes with and without inoculation with PGPB (B+, B-, respectively). **B.** Treatment by trait analysis considering eleven traits and three Treatments (B+, B-, and Delta).

PH: plant height, SD: stalk diameter, RDM: root dry mass, RL: root length, RAD (root average diameter), LRV: lateral root volume, ARV: axial root volume, RA: root Angle, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root.

Table 1. Entry-mean based heritability considering the full model (Eq. 1) and the reduced model for the treatments B+, B-, and Delta (Eq. 2)

Trait[a]	PH	SD	RDM	RL	RAD	LRV	ARV	RA	CHA	SDM	RSR
Full model	0.77	0.67	0.49	0.44	0.43	0.61	0.44	0.34	0.47	0.43	0.51
B+	0.65	0.63	0.35	0.39	0.21	0.54	0.39	0.12	0.32	0.23	0.25
B-	0.63	0.38	0.23	0.31	0.42	0.32	0.34	0.29	0.31	0.20	0.44
Delta	0.08	0.03	0.01	0.13	0.14	0.00	0.14	0.03	0.08	0.00	0.11

[a]PH: plant height, SD: stalk diameter, RDM: root dry mass, RL: root length, RAD (root average diameter), LRV: lateral root volume, ARV: axial root volume, RA: root angle, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root

Population structure and LD decay

The distribution of r^2 declined as the physical distance increased. The LD decay showed different values across chromosomes (Supplementary Figure 8) and ranged from ~175 kb to ~200 kb, considering the r^2 cutoff of 0.10. The first three principal components from PCA analysis showed that the origins of the genotypes (ESALQ and IAPAR) did not form a prominent group. The ADMIXTURE analysis revealed that the population probably presents 18 groups (Supplementary Figure 7).

GWAS

A total of 13,826 SNPs were used for GWAS analysis for all combinations of traits and treatment (Suppl. fig. 9-11). The best good fitness of each model depended on the trait and inoculation. The proportion of the explained variance by significant SNPs ranged from 0.025 to 0.158 (Table 2). There were 30 significant SNPs to treatment B+, 27 for treatment B-, and 8 for Delta. Root-traits had a higher number of significant SNPs. No significant SNPs were found for

RDM and ARV for B+ treatment, and RDM and RA for B-. Contrastingly, for Delta, we found significant SNPs only for PH, SD, and RDM.

Significant SNPs were found in all chromosomes (Fig. 3 B). The SNP CM000784.4_172073449 was significant for the traits RL, SD, and LRV in the treatment B+. For B- and Delta, no overlapping SNPs were found for different traits. On the other hand, the SNP CM007648.1_169026437 was significant for SD in the treatment B+ and Delta. Based on the LD decay of each chromosome, we identified the genes in linkage disequilibrium with the significant SNPs and found 53 overlapping genes associated simultaneously for B+ and Delta (Fig. 3 A). Meanwhile, no candidate genes were shared between B+ and B-, and B- and Delta. For B+ treatment, 55 overlapping genes were found for three different traits.

Figure 2. Manhattan and QQplot for the Delta treatment for (A) PH, (B) RDM, and (C) SD. The highlight SNPs were significant under the permutation test threshold. PH: plant height, SD: stalk diameter, SDM: shoot dry mass

Table 2. Trait, treatment, Marker, chromosome (Chrm), physical position (Posi), minor allele frequency (MAF), p.values, number of genes, and % of the explained variance for each significant SNP from GWAS analysis

Trait[a]	Treat.	Marker	Chrm	Posi	P.value[b]	MAF	NG	r^{2c}	Total[d]
PH	B+	CM000780.4_165053545	4	165053545	5.516	0.211	31	0.015	0.1176
PH	B+	CM000780.4_241977167	4	241977167	6.809	0.236	39	0.020	
PH	B+	CM000781.4_4105099	5	4105099	5.973	0.314	33	0.012	
PH	B+	CM000786.4_145397497	10	145397497	5.403	0.183	64	0.011	
PH	B+	CM007649.1_192589545	3	192589545	6.388	0.193	31	0.015	
PH	B+	CM007650.1_99946782	7	99946782	7.789	0.153	3	0.045	
RA	B+	CM007648.1_230920688	2	230920688	10.085	0.207	21	0.043	0.043
CHA	B+	CM000781.4_59021533	5	59021533	7.827	0.132	10	0.033	0.0646
CHA	B+	CM007647.1_27743555	1	27743555	6.608	0.225	18	0.032	
RAD	B+	CM000780.4_23997789	4	23997789	7.442	0.172	16	0.025	0.067
RAD	B+	CM007647.1_215419190	1	215419190	6.934	0.143	21	0.042	
SD	B+	CM000780.4_235759685	4	235759685	7.666	0.286	19	0.027	0.1584
SD	B+	CM000782.4_2916367	6	2916367	6.052	0.165	25	0.017	
SD	B+	CM007648.1_38816460	2	38816460	6.755	0.264	30	0.030	
SD	B+	CM007648.1_169026437	2	169026437	8.497	0.364	16	0.027	
SD	B+	CM007649.1_31624985	3	31624985	7.306	0.081	22	0.031	
SD	B+	CM007650.1_41779668	7	41779668	8.555	0.375	17	0.026	
RL	B+	CM000784.4_172073449	8	172073449	5.652	0.207	55	0.018	0.018
SDM	B+	CM000780.4_181569268	4	181569268	7.142	0.339	48	0.026	0.0866
SDM	B+	CM000784.4_172073449	8	172073449	5.325	0.207	55	0.017	
SDM	B+	CM007647.1_40109546	1	40109546	5.870	0.483	34	0.026	
SDM	B+	CM007648.1_206189255	2	206189255	5.323	0.396	26	0.017	
LRV	B+	CM000780.4_130864198	4	130864198	6.780	0.165	8	0.022	0.0796
LRV	B+	CM000784.4_172073449	8	172073449	9.494	0.207	55	0.034	
LRV	B+	CM007648.1_205666059	2	205666059	6.141	0.196	21	0.024	
RSR	B+	CM000782.4_92896441	6	92896441	9.767	0.083	8	0.033	0.13

RSR	B+	CM000785.4_154128247	9	154128247	7.560	0.385	43	0.020
RSR	B+	CM007647.1_230383010	1	230383010	5.884	0.064	32	0.036
RSR	B+	CM007648.1_82524308	2	82524308	6.088	0.288	12	0.019
RSR	B+	CM007649.1_65858532	3	65858532	6.157	0.276	13	0.019

[a]PH: plant height, SD: stalk diameter, RL: root length, RAD: root average diameter, LRV: lateral root volume, RA: root angle, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root

[b] $-\log_{10}$(p.value)

[c] the proportion of phenotypic variance explained by SNP.

[d] Sum of the proportion of phenotypic variance explained by SNPs.

Table 2 (continued)**.** Trait, treatment, Marker, chromosome (Chrm), physical position (Posi), minor allele frequency (MAF), p.values, number of genes, and % of the explained variance for each significant SNP from GWAS analysis

Trait[a]	Treat	Marker	Chrm	Posi	P.value[b]	MAF	NG	r^{2c}	Total[d]
PH	B-	CM000784.4_130925210	8	130925210	5.653	0.186	12	0.018	0.0792
PH	B-	CM007647.1_115455586	1	115455586	9.931	0.118	8	0.029	
PH	B-	CM007647.1_297746433	1	297746433	6.310	0.061	37	0.013	
PH	B-	CM007649.1_57358057	3	57358057	6.887	0.319	14	0.019	
CHA	B-	CM007647.1_29786831	1	29786831	7.069	0.368	19	0.025	0.025
RAD	B-	CM007647.1_299457743	8	173246547	9.082	0.050	49	0.028	0.107
RAD	B-	CM000784.4_173246547	9	148920255	5.632	0.306	26	0.016	
RAD	B-	CM000785.4_148920255	9	149343432	6.834	0.124	34	0.024	
RAD	B-	CM000785.4_149343432	1	190289865	6.122	0.163	35	0.183	
RAD	B-	CM007647.1_190289865	1	299457743	6.001	0.361	29	0.020	
SD	B-	CM000782.4_71684361	6	71684361	8.243	0.228	9	0.042	0.1105
SD	B-	CM000785.4_45942495	9	45942495	9.280	0.369	14	0.031	
SD	B-	CM000785.4_142887012	9	142887012	6.567	0.179	22	0.018	
SD	B-	CM007649.1_13703357	3	13703357	5.947	0.436	22	0.020	
RL	B-	CM000781.4_186664447	5	186664447	9.646	0.188	27	0.036	0.036
SDM	B-	CM000780.4_18077503	4	18077503	6.120	0.344	9	0.021	0.1114
SDM	B-	CM007648.1_199939708	2	199939708	7.876	0.283	22	0.027	
SDM	B-	CM007650.1_12165480	7	12165480	8.585	0.065	17	0.039	
SDM	B-	CM007650.1_159938550	7	159938550	7.202	0.418	30	0.025	
ARV	B-	CM000784.4_176063412	8	176063412	6.230	0.138	49	0.029	0.1043
ARV	B-	CM000786.4_143378724	10	143378724	9.735	0.167	42	0.047	
ARV	B-	CM007649.1_149606256	3	149606256	6.080	0.051	23	0.028	
LRV	B-	CM007647.1_96702974	1	96702974	6.372	0.056	11	0.018	0.036
LRV	B-	CM007648.1_34931191	2	34931191	6.305	0.264	23	0.018	
RSR	B-	CM000780.4_238721378	4	238721378	5.663	0.050	15	0.011	0.051

RSR	B-	CM000784.4_109465968	8	109465968	7.059	0.297	15	0.022
RSR	B-	CM007647.1_16710073	1	16710073	6.344	0.254	25	0.018

[a]PH: plant height, SD: stalk diameter, RL: root length, RAD: root average diameter, LRV: lateral root volume, ARV: axial root volume, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root

[b] $-\log_{10}$(p.value)

[c] the proportion of phenotypic variance explained by SNP.

[d] Sum of the proportion of phenotypic variance explained by SNPs.

Table 2 (continued)**.** Trait, treatment, Marker, chromosome (Chrm), physical position (Posi**)**, minor allele frequency (MAF), p.values, number of genes, and % of the explained variance for each significant SNP from GWAS analysis

Trait[a]	**Treat.**	**Marker**	**Chrm**	**Posi**	**P.value[b]**	**MAF**	**NG**	**r^{2c}**	**Total[d]**
PH	Delta	CM000781.4_18267023	5	18267023	5.689	0.293	27	0.039	0.039
SD	Delta	CM000780.4_72636153	4	72636153	7.776	0.302	8	0.03	0.087
SD	Delta	CM000780.4_181664081	4	181664081	5.592	0.286	39	0.015	
SD	Delta	CM007648.1_169026437	2	169026437	5.581	0.363	16	0.017	
SD	Delta	CM007650.1_161417373	7	161417373	6.819	0.307	23	0.025	
RDM	Delta	CM000780.4_243525042	4	243525042	5.643	0.153	57	0.021	0.066
RDM	Delta	CM000781.4_218455951	5	218455951	6.173	0.362	49	0.023	
RDM	Delta	CM007649.1_206185168	3	206185168	5.586	0.052	26	0.022	

[a]PH: plant height, SD: stalk diameter, SDM: shoot dry mass

[b] $-\log_{10}$(p.value)

[c] the proportion of phenotypic variance explained by SNP.

[d] Sum of the proportion of phenotypic variance explained by SNPs.

Figure 3. Venn diagrams (**A**) for overlapping genes for the treatments B+, B-, and Delta, and **(B)** Distribution of the significant SNPs from the GWAS analysis across the chromosomes for eleven traits and treatments (B+, B-, Delta). The bar between the significant SNP indicates the coverage upstream and downstream, based on the LD decay of the respective chromosomes. PH: plant height,

SD: stalk diameter, RDM: root dry mass, RL: root length, RAD: root average diameter, LRV: lateral root volume, ARV: axial root volume, RA: root angle, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root.

Genomic Prediction

Three genomic prediction models were used in order to evaluate the predictive ability for several traits under different treatments. The first model consisted of using a GBLUP model, the second a GBLUP plus the significant SNPs obtained in the GWAS analysis (GBLUP_MAS) as fixed effects. The latter used the genomic additive matrix only the significant SNPs from GWAS (GBLUP_GMS). For the treatment B+, B-, and Delta, the coverage of the significant SNPs from GWAS analysis based on the LD decay was 10.93 MBp, 10.66 MBp, and 24.39 MBp, respectively.

The predictive abilities varied across the traits and treatment (B+, B-, and Delta). For all traits, the treatment Delta was the most difficult to predict; conversely, B+ and B- had similar performance. A similar performance was also observed between GBLUP and GBLUP_MAS. On the other hand, the use of GBLUP_GMS increased predictive ability for several traits and treatments.

Roots-related traits tended to have lower predictive abilities as well as the Delta treatment. For the treatment Delta and LRV, RA, RAD, RSR, the predictive abilities were near zero, regardless of the genomic prediction model. However, GBLUP_GMS increased predictive abilities for ARV, CHA, PH, RDM, RL, SD, and SDM in the Delta treatment.

Figure 4. Predictive abilities for eleven traits under three treatments (B+, Delta, and B-) and three genomic prediction models (GBLUP, GBLUP_MAS, and GBLUP_GMS). PH: plant height, SD: stalk diameter, RDM: root dry mass, RL: root length, RAD (root average diameter), LRV: lateral root volume, ARV: axial root volume, RA: root angle, CHA: convex hull area, SDM: shoot dry mass, RSR: ratio of shoot/root.

DISCUSSION

One of the main challenges in the studies with PGPB is to test the potential biostimulant candidates in field conditions due to its high interaction between host genotype and environmental factors (Rouphael et al. 2018). Our study employed greenhouse conditions, considering the trade-off between the number of genotypes tested and the real environmental conditions (Rouphael et al. 2018). In addition, early trials may be used to evaluate the final performance of the genotypes (Strigens et al. 2012) and assess the population's genetic variance (Wang et al. 2016). Also, many other studies have considered early plant development as a strategy to select for stress tolerance (Grieder et al. 2014; Obeidat et al. 2018).

Recent studies have shown that the host genotype influences the symbiosis with PGPB, suggesting a host's genetic control (Wintermans et al. 2016; Proietti et al. 2018; Vidotti et al. 2019a). Moreover, the host heterosis plays an important role in shaping bacterial and fungal rhizosphere community composition (Wagner et al. 2020). To the best of our knowledge, this is the first study to evaluate the symbiosis between a synthetic population of PGPB and a public tropical maize association panel. Our results revealed that the synthetic population of PGPB showed biostimulant effects in the panel. Also, the PGPB impacted root architecture, and for most traits, influenced the phenotypic variation (Vidotti et al. 2019b). The PGPB benefits in roots have been associated due to the ability of the PGPB to produce plant hormones, such as indole-3-acetic acid (IAA) (Remans et al. 2008), ethylene (Barnawal et al. 2012), abscisic acid (Belimov et al. 2014), gibberellin (Khan et al. 2014), and cytokinins (Liu et al. 2013; Khan et al. 2014).

The strong correlation between root traits and PH, SD, and SDM confirms its importance for absorption and nutrient supply to biomass synthesis. Also, the correlation between root architecture traits revealed a mutual association between them, although GWAS analysis revealed that probably different genomic regions control them. Differential correlation between traits for treatments B+ and B-, and phenotypic variance may indicate that the PGPB can modulate the plant and root architecture. The association between RDM and SD and Delta treatment reveals the importance of these traits for the response to PGPB. Hence, seven significant SNPs were found for RDM and SD in the Delta treatment.

The effects of genotype and treatments were significant for most of the traits revealing that the PGPB promoted growth in the population, which has genetic variability. On the other hand, we didn't find significant effects for the interaction between genotype and treatment. The lack of interaction between genotype and treatment may be due to several factors such as small or absence of phenotypic plasticity in the population, early evaluations, and/or nitrogen-limited conditions limited growth in the B- treatment, making it difficult to assess the interaction. Although, we found different SNPs in linkage disequilibrium for the treatment B+ and B- for several traits. The differential association between SNPs and treatments may suggest that other genomic regions are responsible for growth in the presence or absence of PGPB. Furthermore, SNPs associated with the Delta treatment highlight the genetic basis for the response of PGPB. At the same time, the GWAS results reported here for B+, B-, and Delta must be interpreted with caution since the traits studied are expected to be polygenic with small effects, and the reproducibility of the GWAS is expected to be low in this cases (Bian et al. 2014). Also, B+ and B- differences may be functional and not directly related to the bacteria.

The small heritability for Delta treatment reveals the challenges to evaluate the response to the PGPB. Nevertheless, we found eight significant SNPs associated f0r three different traits. Overlapping SNPs for the treatments B+ and Delta and others for B+ may indicate the presence of pleiotropic effects or linkage disequilibrium. Unfortunately, the magnitude of the LD decay in our population limits the interpretation of our analysis due to the size of the cover of each SNPs, making it difficult to find possible candidate genes for the trait. However, the overlapping genes in the treatments B+ and Delta and from the different traits for B+ can be used as possible candidate genes for further investigation of candidate genes for symbiosis between plant growth-promoting bacteria and maize.

Several studies have been using GWAS and genomic prediction in order to understand the genetic architecture of many traits (Wallace et al. 2016; Galli et al. 2020). Our results revealed that it is possible to increase the predictive ability of several traits when using a subset of SNPs representing important genomic regions, even if the trait heritability is low. The increase in predictive ability, especially in the Delta treatment, when considering only a small part of the genome to

compute the GRM, may endorse the importance of these genomic regions for the response to PGPB. Also, these genomic regions can be explored via plant breeding for selection.

Root-traits information seems to be a key to breeding for resilience (Lombardi et al. 2021). Besides the difficulty of phenotyping the roots, a large amount of information generated and how to use this information in the decision-making process is still not fully comprehended. The evaluation of roots is usually laborious due to the need to wash the roots and evaluating them using visual scores (Trachsel et al. 2011) or image analysis (Seethepalli et al. 2021). In our work, we presented a shovelomics pipeline in order to evaluate, analyze, and apply the root traits in the genetic architecture studies and their possible application into plant breeding programs. The predictive ability of RDM, SDM, and most of the traits in the Delta treatment substantially increased when we used the GRM with only the SNPs in disequilibrium with the above and underground traits highlighting the importance of phenotyping these traits (Yonis et al. 2020).

Our results corroborate the hypothesis that multiple genes with small effects are responsible for the response to the PGPB (Cotta et al. 2020). Furthermore, the genetic basis of the response to the PGPB can be used for plant breeding programs to maximize the symbiosis between tropical maize and PGPB and increase plant resilience against biotic and abiotic stress. Also, we suggest that further studies should be conducted in order to validate the SNPs and the genes responsible for the interaction between tropical maize and PGPB.

CONCLUSION

Despite the limitations, our study contributed to understanding the role of the host genotype in the symbiosis with PGPB. In tropical maize, it is controlled by many genes and has a quantitative inheritance. Furthermore, our tropical maize germplasm showed a significant genetic variability to the symbiosis with PGPB, being a good source of alleles for plant breeding programs to develop more resilient genotypes for tropical agriculture.

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