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Genome-Wide Selection for tropical maize root traits under conditions of nitrogen and phosphorus stress

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ABSTRACT. The objective of this study was to verify the accuracy of the Genome-Wide Selection (GWS) method in tropical maize breeding for root traits under conditions of nitrogen and phosphorus stress. Forty-one single-crosses were evaluated in two experiments. The first experiment considered low nitrogen availability, and the second experiment considered low phosphorus availability. A randomized block design with two replicates was used. The lateral and axial root lengths were measured using WinRhizo software. The analysis of deviance was calculated using the Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) method. Eighty microsatellite markers were used to genotype the estimation population. The Random Regression method was used to analyze the GWS (RR-BLUP/GWS) data. The gains per unit time of the GWS and the phenotypic selection method were compared, as the standard phenotypic selection methods were considered to be the Recurrent Selection. The GWS accuracy was higher than the phenotypic selection accuracy for all of the traits evaluated. Thus, the GWS method may significantly increase the genetic gains for root traits that are obtained in tropical maize breeding programs for nutritional stress conditions.

Keywords: marker-assisted selection, recurrent selection, abiotic stresses.

Seleção Genômica Ampla para caracteres de raiz em milho tropical em condições de estresse de nitrogênio e fósforo

RESUMO. O objetivo deste estudo foi verificar a acurácia do método da Seleção Genômica Ampla (GWS) no melhoramento de milho tropical para caracteres de raiz em condições de estresse de nitrogênio e fósforo. Quarenta e um híbridos simples foram avaliados em dois experimentos. O primeiro experimento considerou baixa disponibilidade de nitrogênio e o segundo baixa disponibilidade de fósforo. Para isto, foi usado o delineamento de blocos casualizados com duas repetições. Os comprimentos de raiz lateral e axial foram medidos utilizando o software WinRhizo. As análises de deviance foram realizadas pelo método da Máxima Verossimilhança Restrita / Melhor Preditor Linear Não-viesado (REML/BLUP). Oitenta marcadores microssatélites foram utilizados para determinar o genótipo da população de estimação. O método de regressão aleatória foi utilizado nas analises de GWS (RR-BLUP/GWS). Os ganhos por unidade de tempo da GWS e dos métodos de seleção fenotípica foram comparados, sendo que como método padrão de seleção fenotípica foi considerado a seleção recorrente. A acurácia da GWS foi maior do que a acurácia da seleção fenotípica para todas as características avaliadas. Assim, o método GWS pode aumentar significativamente os ganhos genéticos para características de raízes que são obtidos em programas de melhoramento de milho tropical condições de estresse nutricional.

Palavras-chave: seleção assistida por marcadores, seleção recorrente, estresses abióticos.

Introduction

The Genome-Wide Selection (GWS) method simultaneously predicts (without the use of significance tests for individual markers) the genetic effects of a large number of molecular markers that are distributed throughout the whole genome of an organism in order to capture the effects of all of the loci and explain the entire genetic variation of a quantitative trait (MEUWISSEN et al., 2001). After these markers are identified, their effects are estimated from phenotypic data that are obtained from a population that is known as the estimation population. Once the effects are estimated, they are tested in a validation population. After this step, the markers that explain most of the genetic variance of a trait are selected. Thus, this information is effectively incorporated into the selection stage of the breeding program (RESENDE, 2008).

Efficient root systems enhance the relationship between the resources acquired and used for obtaining new resources and maintaining themselves (ZHU et al., 2005). Several studies have investigated the relationship between root traits and the components of nutritional use efficiency in maize (BONIFAS; LINDQUIST, 2009; DOVALE et al., 2012; LIU et al., 2004; ZHU et al., 2005). Plants typically respond to a nutrient deficiency by increasing the total length of both axial and lateral roots (CHUN et al., 2005). Thus, studies investigating root morphology and plasticity when faced with environmental changes may be relevant for understanding the nutritional use efficiency of plants and, consequently, for helping select genotypes that are more tolerant to abiotic stresses (RUTA et al., 2010).

The phenotype-based evaluation and selection of root traits uses methods that destroy the plant, which, depending on the genetic structure of the population, do not allow the use of the plants that were identified as superior in hybridizations. Consequently, there is a reduction in the potential gains that are obtained from the selection. In addition, some conventional breeding methods, such as recurrent selection, are very laborious and have shown low selection gains per unit time, often becoming uneconomical.

These facts can be mitigated with the use of GWS, which allows the early identification of superior genotypes without using destructive methods, thereby increasing the gains from selection and reducing the interval between generations. Prediction and selection may be performed at very early plant stages in this method, thus accelerating the breeding process. Additionally, the prediction tends to be more accurate because it considers the actual genetic relatedness of the evaluated individuals, rather than the mathematically calculated expected average relatedness. Thus, the GWS enables a direct early selection because it acts on the genes that will be expressed in adulthood earlier than traditional early selection, which is indirect and acts (via phenotypic assessment) on the genes that are activated at an early age, hoping that it informs about its expression in adulthood (RESENDE, 2008).

Given the above, the objective of this study was to verify the accuracy of the GWS method when used in tropical maize breeding for root traits under conditions of nitrogen and phosphorus stress.

Material and methods

Genetic material and experimental design

Two sources of germplasm were used in these experiments. The first consisted of seven inbred lines without any genetic variability in phosphorus use efficiency (PUE), but with differing values of nitrogen use efficiency (NUE) (Group I). The second group of seven inbred lines did not contain any genetic variability in terms of NUE, but differed in terms of PUE (Group II). Forty-one single-crosses were obtained by intergroup factorial mating (unbalanced) of the lines in these groups. These crosses were made reciprocally and involved the mixing of seeds of the same combination.

The 41 single-crosses were evaluated in two experiments. The first experiment considered low nitrogen availability (LN), and the second experiment considered low phosphorus availability (LP). A randomized block design with two replicates was used in these experiments. The experiments were conducted in a greenhouse (20°45'14"S, 42°52'53"W) at the Federal University of Viçosa, Brazil in October 2010.

The seeds were pre-germinated in a box with individual germination cells, with three seeds per inbred line. Soon after emergence, one seedling from each inbred line was transplanted to a cylindrical 4 dm^3 PVC pot (10 cm diameter x 50 cm height). The substrate for the N experiment was prepared by mixing sand and vermiculite in a 1:1 ratio using the methodology described by Walk et al. (2006). For the P experiment, a mixture of 50% sand, 37.5% vermiculite and 12.5% soil with very low phosphorus was used. The soil was used to absorb the phosphorus and prevent it from being readily available to the plant.

The nutrient solution was replenished every two days starting on the seventh day after seedling emergence. The solution described by Chun et al. (2005) was used in the N experiment. Low P availability was simulated in the P experiment by adding only 34 mg dm^{-3} of phosphorus in the form of triple superphosphate. The other nutrients were supplied by the nutrient solution without any added phosphorus addition.

The seedlings were harvested at the vegetative stage with six completely expanded leaves (V6), which occurred 28 days after sowing. The seedling shoots were separated from the root system and stored in containers with water and alcohol solution (3:1).

The plant root systems were evaluated by image analysis using WinRhizo Pro 2009a software (Basic, Reg, Pro & Arabidopsis for Root Measurement) coupled to an Epson Perfection V700/V750 scanner that was equipped with additional light (TPU) and a resolution of 400 dpi, as described by Bouma et al. (2000). The lateral and axial root lengths were extracted from all diameter classes (d), and the

lateral root lengths (L_{LAT}) with a diameter less than or equal to 0.5 mm and axial root lengths (L_{AXI}) with a diameter greater than 0.5 mm were selected by WinRhizo, as described by Trachsel et al. (2009).

Analysis of deviance

The data from each experiment were subjected to analysis of deviance using the Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) method described by Resende (2002):

$$y = Xr + Zm + Wf + Tc + e$$

where:

y is the vector of phenotypic means of single-crosses; r is the vector of replicate effects (assumed to be fixed), summed up in a general average; m is the vector of parent effects from Group II (assumed to be random), where $m \sim N(0, M)$ and $M = I\sigma_m^2$; f is the vector of parent effects from Group I (assumed to be random), where $f \sim N(0, F)$ and $F = I\sigma_f^2$; c is the vector of specific combining ability between the parents of Group I and the parents of Group II (assumed to be random), where $c \sim N(0, C)$ and $C = I\sigma_c^2$; and e is the vector of errors, where $e \sim N(0, R)$ and $R = I\sigma_e^2$. X, Z W and T are incidence matrices that relate the effects of r, m, f, and **c**, respectively, to vector *y*.

The analyses were performed using SELEGEN-REML/BLUP software (RESENDE, 2007a).

Genotyping the estimation population

Eighty microsatellite markers (SSR) were used for genotyping. These markers were chosen for their informational content, location on chromosomes and, mainly, for their association with agronomic traits and abiotic stresses tolerance (MAIZEGDB, 2009).

The genotyping was only performed on the 14 parent lines, which had more than seven inbred generations. Thus, it was possible to extrapolate the marker results to single crosses. Only those markers that showed a frequency greater than 5% in the single crosses were considered.

Genome Wide Selection (GWS)

The Random Regression with BLUP method (RR-BLUP) was applied to the genome-wide selection (RR-BLUP/GWS) method to analyze the data, as described by Guo et al. (2012):

$$y = Xb + Zh + e$$

where:

y is the vector of phenotypic means of single crosses, b is the vector of fixed effects, h is the vector of marker effects (random) and e is the vector of errors (random). X and Z are the incidence matrices for b and h, respectively. Z has values of 0, 1 and 2 depending on the number of markers. The mean and variance structure is defined by $h \sim N(0, G)$; $E(y = Xb)$; $e \sim N(0, R = I\sigma_e^2)$;

$Var(y) = V = ZGZ' + R$; $G = I\sigma_A^2 / n$.

The mixed model equation for predicting h by RR-BLUP/GWS is:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \dfrac{\sigma_e^2}{\sigma_A^2 / n} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{h} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

where:

σ_A^2 is the total additive genetic variance of a trait, σ_e^2 is the residual variance and n is the weighted number of markers obtained by $n = \left[2\sum_i p_i(1 - p_i) \right]$, where *pi* is the frequency of marker *i*; $\sigma_e^2 / (\sigma_A^2 / n) = (1 - h_{Am}^2) / (h_{Am}^2 / n)$; and h_{Am}^2 is the narrow sense heritability from the single-cross average, which is estimated by $h_{Am}^2 = \dfrac{r.h_A^2}{1 + (r-1)h_A^2}$, where r is the number of replicates. Each locus is considered to explain $\dfrac{1}{n}\sigma_A^2$ in this method. In other words, equal parts of the genetic variance are assigned to all loci. The global genomic breeding value of the individual j is given by $VGG = \hat{y}_j = \sum_i Z_i \hat{h}_i$.

The RR-BLUP/GWS procedure also allows for the identification of those markers that have major effects, with the goal of performing the analysis with smaller subgroups of markers to determine how many and which of them maximize selection accuracy. Thus, the entire set of markers was first analyzed in all of the evaluated individuals (the complete estimation population). Nine marker subgroups that consisted of 214 (all markers), 150, 100, 50, 25, 10, 5, 2 and 1 marker(s) were generated from these results. The formation of these subgroups was based on the marker effect (in modulus) obtained in the initial analysis, i.e., considering all 214 markers. From these values, the

markers with lesser effects were sequentially removed from the analysis until the one marker with the highest effect remained, forming the subgroup.

These results were cross-validated by resampling groups of individuals with the Jacknife procedure (HELTSHE; FORRESTER, 1983). In this process, the GWS was evaluated in each subgroup analyzed to calculate the correlation between the genetic value predicted by this method and the phenotype observed in individuals. This correlation is known as the predictive accuracy ($r_{y\hat{y}}$), which is calculated by multiplying the selection accuracy ($r_{g\hat{g}}$) with the square root of individual heritability (h) ($r_{y\hat{y}} = r_{g\hat{g}}h$) (RESENDE, 2008).

The GWS was also compared to the phenotypic selection method in terms of the gain per unit time. For this, the Recurrent Selection (RS) and Reciprocal Recurrent Selection (RRS) models were considered the standard phenotypic selection models. In the case of RS, the formation of a unique population originating from the free crossing of different inbred lines was considered. Two hundred half-siblings were obtained from this population for evaluation, and 200 S_1 siblings were obtained for recombination. These progenies were evaluated in an experiment that was performed in a plot containing 25 plants and replicated twice. A total of 10,000 plants were evaluated. In addition, we considered the selection of 10% superior progenies. For the RRS analysis, the formation of two base populations that originated from the free crossing of different lines within each heterotic group (HG) was considered. Two hundred inter-population half-siblings were obtained from these HGs for evaluation, and 200 S1 progenies within each HG for were obtained for recombination. These progenies were evaluated in an experiment that was performed in a plot containing 25 plants and replicated twice. A total of 10,000 plants were evaluated. In addition, we considered the selection of 10% higher inter-population single crosses.

That same population of 10,000 plants was considered for the intra-population GWS, with the 200 best individuals selected before flowering and recombination (i.e., a 2% selection intensity at the level of individual, with coincidence in the units and generations of evaluation and recombination). The selection for the inter-population GWS was conducted at the same intensity and effective size as that of the intra-population; however, the selection was divided within each HG. Thus, if the phenotypic and genetic variances were equally divided in the HG, the gains from the RS and RRS selections would theoretically be equal.

The gains in selection in additive genetic standard deviations were estimated by $GS = k.r_{g\hat{g}}$, where k=1.76 for the RS and RRS methods and 2.42 for the GWS, and $r_{g\hat{g}}$ is the selective accuracy (estimated by cross-validation for GWS), which is obtained by $\sqrt{1/4h_{Am}^{2*}}$ for RS and RRS, where $h_{Am}^{2*} = \dfrac{r(1/2)h_A^2}{1+(r-1)(1/4)h_A^2}$ and r is the number of replicates. This value of ¼ for the heritability refers to the types of progeny that were considered for evaluation and recombination. The relative efficiency of the GWS method in relation to RS and RRS was obtained for each trait by using these estimates as follows: $RE_{GWS(\%)} = \dfrac{GS_{GWS}.t_{RS;RRS}}{GS_{RS;RRS}.t_{GWS}}.100$, where GS is the gain of selection estimated in additive genetic standard deviations for each method (GWS, RS and RRS); t is the time (years) to complete each method, with t =1,5 for RS and RSS and 1,0 or 0,5 for GWS, considering the possibility of one or two cycles per year, respectively.

The analyses were performed using SELEGEN Genômica RR-BLUP/GWS software (RESENDE, 2007b).

Results and discussion

The analysis of deviance for the two experiments showed one or more sources of genetic variation with significant differences for all traits. This result indicates the existence of genetic variability, which allows for the selection of and genetic gains in maize root traits and, consequently, a better response to the effects of abiotic stresses. The coefficients of variation for the root traits were high when compared to other traits in maize (FRITSCHE-NETO et al., 2012). However, they are within the acceptable levels for this type of study (FRITSCHE-NETO et al., 2010) (Table 1).

Two hundred fourteen alleles with a frequency greater than 5% were identified in the hybrid population, with an average of 2.68 markers per locus and a range of two to five markers.

The traits exhibited great variation in the number of markers that maximized the predictive and selection accuracy of the GWS, with values ranging from two markers for L_{AXI} in LP to 25 markers for L_{AXI} in LN (Table 2). Normally, the predictive accuracy decreases with an increasing number of markers (RESENDE, 2008). This effect was observed in this work, as the predictive accuracy was very low when all 214 markers were considered.

In other words, increasing the number of markers may not linearly increase the accuracy of the RR-BLUP/GWS method (Tables 1 and 2). Decreasing the number of markers used in the GWS from the point of maximal accuracy also reduced the accuracy and explained a proportion of the genetic variance (RESENDE, 2008). Thus, it is possible that a marker-assisted selection (MAS) that is based on one or a few markers provides an efficiency that is significantly lower than that of the GWS. Thus, it is essential to select the optimal number of markers for each trait of interest to maximize the accuracy in the validation population.

The markers explained 48, 13, 68 and 49% of the genetic variation in the LLAT and LAXI traits under LN and LP conditions, respectively. These markers also provide high accuracy for the most selective of these traits (69, 36, 83 and 70%, respectively) (Table 2). Zhao et al. (2011) observed similar values for grain yield and maize grain moisture, as did Guo et al. (2012) for days to silking, days to anthesis and anthesis silking interval.

Theoretically, the probability of finding markers in linkage disequilibrium with traits increases with larger populations and more markers (FLINT-GARCIA et al., 2003). However, it was possible to obtain good results for all of the traits evaluated (even with the small effective size and number of markers used in this work), and the maximal GWS accuracy was higher than the phenotypic accuracy (Table 2). This increase is due to the use of a real relationship matrix; in other words, a relationship that is based on the markers that individuals have and not the traditional methods that are based on mathematical expectation (RESENDE, 2008).

The fact that a small number of markers explained the majority of the genetic variation or maximized the selective accuracy is interesting from a practical standpoint because a small group of pre-selected markers may be used for selection in breeding populations. Thus, it is possible to reduce the interval between selection cycles or use non-destructive evaluation methods that increase the genetic gains for root traits.

Table 1. Likelihood Ratio Test (LRT) values for the general combining ability (GCA) effects of groups I (GI) and II (GII) and the specific combining ability (SCA) effects between these two groups, estimates of average, heritability nurse sense (h_a^2) and the coefficients of variation (CV) for the lateral (LLAT) and axial root lengths (L_{AXI}) under low N (LN) and phosphorus availability (LP).

Effects	LN		LP	
	L_{LAT}	L_{AXI}	L_{LAT}	L_{AXI}
GCA (GI) [1]	0.32 [NS]	1.89 [NS]	1.77 [NS]	0.48 [NS]
GCA (GII) [1]	3.44[*]	6.9[***]	8.30[***]	5.32[**]
SCA GI x GII [1]	1.21 [NS]	1.58 [NS]	3.88[**]	5.82[**]
h_a^2	0.14	0.24	0.24	0.13
Average	19.74	8.02	29.19	11.30
CV (%)	24.63	23.67	14.87	17.71

[1]Values obtained from the Likelihood Ratio Test (LRT); [***]p = 0.01, [**]p = 0.05, [*]p = 0.10, e [NS] not significant after performing a χ^2 test with 1 df.

Table 2. Estimated predictive accuracy ($r_{y\hat{y}}$) and selection accuracy ($r_{g\hat{g}}$) of the GWS, with the genetic proportion of the genetic variance explained by markers (%Vg) that considered different amounts of markers (subgroups) in the validation analysis of the GWS and the phenotypic accuracy (r_{phe}) of the lateral (L_{LAT}) and axial root lengths (L_{AXI}) under low N (LN) and phosphorus availability (LP).

Trait		Estimative	Number of markers considered								
			214	150	100	50	25	10	5	2	1
LN	L_{LAT}	$r_{y\hat{y}}$	0.13	0.21	0.27	0.31	0.36	0.41	0.39	0.38	0.41
		$r_{g\hat{g}}$	0.27	0.43	0.54	0.62	0.72	0.83	0.78	0.75	0.82
		r_{phe}	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
		%Vg	0.07	0.18	0.30	0.39	0.51	0.68	0.61	0.56	0.67
	L_{AXI}	$r_{y\hat{y}}$	0.38	0.39	0.40	0.43	0.43	0.30	0.19	0.05	0.11
		$r_{g\hat{g}}$	0.61	0.62	0.63	0.69	0.69	0.49	0.31	0.09	0.17
		r_{phe}	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
		%Vg	0.37	0.39	0.40	0.47	0.48	0.24	0.10	0.01	0.03
LP	L_{LAT}	$r_{y\hat{y}}$	0.22	0.23	0.24	0.22	0.28	0.44	0.37	0.08	-0.41
		$r_{g\hat{g}}$	0.35	0.37	0.39	0.34	0.45	0.70	0.60	0.13	-0.66
		r_{phe}	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
		%Vg	0.13	0.14	0.15	0.12	0.20	0.49	0.36	0.02	0.43
	L_{AXI}	$r_{y\hat{y}}$	0.09	0.12	0.11	0.09	0.15	0.17	0.17	0.17	0.06
		$r_{g\hat{g}}$	0.18	0.26	0.24	0.19	0.31	0.35	0.35	0.36	0.12
		r_{phe}	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
		%Vg	0.03	0.07	0.06	0.03	0.10	0.12	0.13	0.13	0.01

Considering the predicted gain estimates from one cycle of GWS per year, it is possible to increase the selection efficiency relative to the traditional Recurrent Selection and Reciprocal Recurrent Selection methods for the L_{LAT} and L_{AXI} traits under LN and LP to 927, 599, 608 and 419%, respectively (Table 3). When the possibility of two cycles per year was considered, the values increased to 2992, 1934, 1960 and 1350%, respectively.

Table 3. Estimates of the gains with selection (GS) in the additive genetic standard deviations for each method considered and the relative efficiency (RE) of Genome-Wide Selection (GWS) with one (GWS 1) or two (GWS 2) cycles per year, relative to the Recurrent Selection (RS) and Reciprocal Recurrent Selection (RRS) methods for lateral (L_{LAT}) and axial root length (L_{AXI}) under low N (LN) and N availability (LP).

Estimates	Method	Trait			
		LN		LP	
		L_{LAT}	L_{AXI}	L_{LAT}	L_{AXI}
GS	RS/RRS	0.32	0.42	0.42	0.31
	GWS	2.00	1.67	1.70	0.87
RE (%)	GWS 1	927	599	608	419
	GWS 2	2992	1934	1960	1350

The significant increase in genetic gain with GWS is due to several factors. Of these, the coincidence between the units and the cycles of selection and recombination in just one generation in the field deserves mention. In addition, selection is performed on individuals of both sexes and does not need to be performed in the ideal environment for evaluation. Thus, there is an increase in the selective accuracy and gain per unit time of the breeding program.

Conclusion

The Genome-Wide Selection method may significantly increase the genetic gains in maize root trait breeding programs that investigate nutritional stress conditions.

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