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Genomic prediction in grain sorghum under low-P conditions using gene-specific molecular markers

Crislene Vieira dos Santos
Doctor Scientiae

VIÇOSA - MINAS GERAIS
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CRISLENE VIEIRA DOS SANTOS

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Thesis submitted to the Genetics and Breeding Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Aluizio Borem de Oliveira

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Agradeço a Deus, à minha família e aos meus amigos.

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Thank you, God, for all the times you gave me strength and made me wise. Thank you for keeping me strong in my path and confident that this was the right way. Thank you so much for putting in my way people who supported me and renewed my faith.

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“My dreams are bigger than my fears”.
(Viola Davis)

ABSTRACT

SANTOS, Crislene Vieira dos, D.Sc., Universidade Federal de Viçosa, December, 2024. **Genomic prediction in grain sorghum under low-P conditions using gene-specific molecular markers**. Adviser: Aluizio Borem de Oliveira.

Genomic prediction presents a powerful opportunity for enhancing sorghum breeding programs, enabling the selection of hybrids that achieve high yields even in low phosphorus (P) environments. This paper investigates genomic selection for predicting sorghum hybrid performance by evaluating 110 R parent lines using molecular markers crossed with two female lines to produce 188 hybrids tested over two years (2021 and 2022) in low phosphorus conditions. Results showed high accuracy in selecting non-tested hybrids. This innovative approach not only identifies the most promising hybrids but also efficiently filters out those that lack tolerance or responsiveness to challenging conditions. The accuracy of genomic predictions for grain yield exceeds 0.6 for critical traits such as Grain Yield, Plant Height, 1000 Grain Weight, and Flowering Date. Notably, the prediction for Grain Yield has demonstrated exceptional reliability, with an accuracy of 0.67, and the predicted values closely match real-world field data. By adopting genomic predictions, we can significantly advance breeding strategies and improve crop performance in adverse conditions.

Keywords: Genomic prediction; Grain Yield; Phosphorus efficiency of uptake

RESUMO

SANTOS, Crislene Vieira dos, D.Sc., Universidade Federal de Viçosa, dezembro de 2024. **Predição genômica em sorgo granífero sob condições de baixo P usando marcadores moleculares específicos de genes**. Orientador: Aluizio Borem de Oliveira.

A predição genômica é uma ferramenta importante para o avanço dos programas de melhoramento de sorgo, permitindo a seleção de híbridos que alcançam alta prodtividade de grãos, e permitindo predizer o valor genético daqueles que ainda não foram testados em campo. Este artigo investiga a seleção genômica para predizer o desempenho de híbridos de sorgo avaliando suas respectivas linhagens parentais (110 linhagens R) que foram previamente selecionadas por marcadores moleculares. Essas linhagens R foram cruzados com duas linhagens A para produzir 188 híbridos testados ao longo de dois anos (2021 e 2022) em condições de baixo teor de fósforo. Os resultados mostraram alta precisão na seleção de híbridos não testados. Esta abordagem não só identifica os híbridos mais promissores, mas também filtra os de baixa produtividade que podem ser descartados. No presente trabalho a precisão das predição genômicas excede 0,6 para características críticas, como rendimento de grãos, altura da planta, peso de 1.000 grãos e data de floração. A predição do rendimento de grãos demonstrou confiabilidade, com acurávia depredição de 0,67, e os valores previstos correspondem de perto aos dados de campo. Adotando a predição genômica pode-se significativamente avançar os programas de melhoramento de sorgo selecionando hybridos de melhor performance em condições adversas.

Palavras-chave: Predição genômica; Produtividade de grãos; Eficiência de aquisição de fósforo

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GENERAL INTRODUCTION

Sorghum is consumed by more than 500 million people, in more than 30 countries, with emphasis on the African and Asian continents (Hariprasanna and Rakshit, 2016). In countries where sorghum consumption for human food is more common, the cereal is responsible for up to 70% of daily caloric intake (Xiong et al., 2019). The establishment of sorghum on the market is due to its yield performance in regions and conditions in which other crops, used for the same purpose, do not present the same profitability to the grower.

Sorghum originated in northeast Africa, near the equator, and many varieties are sensitive to day length and temperature. To be cultivated in the Americas (United States, Mexico, Argentina, and Brazil), sorghum underwent a conversion program. This program aimed to make the plant insensitive to photoperiod, select shorter plants, and increase grain yield (Smith; Frederiksen, 2000; Rooney, 2004). Due to increased research, Sorghum has become the world's fifth most cultivated cereal after wheat, corn, rice, and barley. It is now grown on all continents, with a strong presence in semi-arid regions with irregular rainfall. In Brazil, it is mainly cultivated in the Cerrado areas, which offer better conditions than the semi-arid regions.

In Africa and Asia, sorghum is widely used for human food. In the Americas, sorghum is most used for livestock in the form of feed and silage. In Brazil, grain sorghum finds excellent climatic conditions for development. Planting area of the crop is concentrated in the Cerrado region in succession to summer soybeans (February), in the South in summer plantings (November), and in the Northeast in plantings at the beginning of the rains (February/March). All planting is done under rainfed conditions.

The highest productivity is obtained by being assertive in choosing cultivars adapted to each region, managing weeds, diseases, pests, and fertilizing (Balemi et al., 2012; Batista et al., 2019; Bernardino et al., 2020). Although, sorghum is frequently listed as a second choice, for the farmers, mainly because, when planted until early February, the corn has more attractive prices and has higher yields. The sorghum is not a competitor against corn, but a smart strategy when the environmental conditions are not suitable for corn cultivation (Menezes et al., 2019). Sorghum is a strategic crop for areas with low phosphorus availability, being efficient in exploring the soil, via its robust root system, and increasing the profitability of the land with lower costs (Bernardino et al., 2019).

Brazilian soils have low phosphorus, what is a significant challenge for agriculture, especially since phosphorus is one of the essential nutrients for plant growth, playing a crucial role in root development, photosynthesis, seed formation, and grain filling (Silva et al., 2021). Phosphorus is, therefore, one of the limiting nutrients for agricultural productivity in Brazil, especially in some regions with low fertility soils.

The impact of soils with low phosphorus content on Brazilian agriculture can be observed in several aspects, such as reduced plant growth, decreased productivity, and increased production cost due to the need for additional fertilization. Phosphorus in soil can exist in forms that are not available, such as iron and aluminum phosphates, especially in acidic soils and in soils with low organic matter contents. Even if there are apparent amounts of phosphorus in the soil, its availability to plants is limited. Phosphorus deficiency in Brazilian soils has a significant impact on agriculture, resulting in reduced productivity, increased fertilizer costs, and a greater vulnerability of crops to nutritional deficiencies. Acidic soils poor in organic matter, common in Brazil, are the main responsible for the immobilization of phosphorus, making it less available to plants. Sorghum genetic improvement is one of the fundamental strategies to improve the efficiency of phosphorus use by plants, contributing to sustainability and increased productivity.

The efficiency of sorghum in uptaking phosphorus (P) in the soil is a crucial factor in determining its growth and productivity, since phosphorus is an essential nutrient for root development, seed formation, and energy processes within the plant. Phosphorus is often present in soil in forms that are not available to plants, such as iron and aluminum phosphates in acidic soils or calcium phosphates in basic soils. Therefore, sorghum's ability to acquire phosphorus efficiently depends on several characteristics, genetically controlled, mainly by two genes, SbMATE and PSTOL1.

The first gene associated with aluminum (Al) tolerance in sorghum is the SbMATE gene (Magalhães et al., 2007). Researchers studied Al tolerance in an F2:3 population derived from a cross between the sorghum lines SC283 (tolerant) and BR 007 (sensitive). The findings indicated that the genetic improvements in sorghum were consistent with the presence of a gene conferring Al tolerance that accounts for approximately 80% of the phenotypic variation for this trait (Magalhães et al., 2004; Magellan; Guimarães, 2008).

The SbMATE gene was isolated through positional cloning at the AltSB locus on sorghum chromosome 3, which is primarily responsible for the Al tolerance phenotype (Magalhães et al., 2004, 2007). Notably, the SbMATE gene shows enhanced expression at the root apex of tolerant genotypes and is induced in the presence of toxic Al. This gene enables sorghum to tolerate Al by facilitating the release of citrate into the rhizosphere of tolerant plants. Citrate forms a stable, non-toxic complex with Al ions, mitigating their harmful effects on plant roots.

The sorghum balances the market demand for grain quality and maximizes the use of P converted in grain, reducing phosphorus dependence. That is also a clear sign that agriculture needs strategies to reduce phosphorus dependence, selecting genotypes with higher efficiency of P use, using breeding tools, like genomic selection and prediction.

Genomic selection (GS) and Genomic Prediction (GP) are trustful tools for selection. In breeding programs, the selection based on genetic values can help develop genotypes with mechanisms to uptake P_2O_5 more efficiently. The GS and GP have been fully applied in many animal and plant breeding programs, showing prediction accuracy and a considerable correlation with heritability (h^2) (Los Campos et al., 2013, Fernandes et al., 2018). When a particular trait has low h^2, indirect or multi-trait GS can be applied to take advantage of correlated traits with higher h^2, thus improving the target trait (Mrode 2014). The benefits of multi-trait GS over single-trait GS have been reported in simulated (Calus and Veerkamp 2011) and real data (Jia and Jannink 2012; Schulthess et al. 2016).

A previous study used single-trait genomic selection to predict grain yield in a sorghum panel and found that a model including plant height, stalk number, and lodging could explain a large portion of the phenotypic variation in grain yield ($R^2 = 0.63$). Indirect genomic selection using these traits like root system traits can be applied to cut the time of selection of sorghum lines with morphological mechanisms associated with grain sorghum yield (Lopéz-Arredondo et al., 2014; Burks et al. 2015).

The present study aimed to select sorghum lines and hybrids with competitive grain yield and the highest efficiency in phosphorus acquisitions under soils with low P availability through genomic prediction.

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CHAPTER 1

GENE-SPECIFIC MOLECULAR MARKERS ASSOCIATED WITH TOLERANCE TO ABIOTIC STRESSES IN GRAIN SORGHUM.

ABSTRACT

Aluminum toxicity and low phosphorus availability can decrease sorghum yield by more than 50%. Therefore, some genotypes have demonstrated the ability to grow and produce reasonable grain yield under low phosphorus availability. Hybrid performance is determined by combining different alleles at various loci associated with the desired trait. Recent studies suggest that new methods can predict the performance of a hybrid by analyzing the genetic value of its inbred parents. Such predictions can significantly reduce the number of hybrids that need to be evaluated in the field, thus decreasing the costs of production and testing of many crosses. The present paper aimed to study genomic selection to predict the sorghum hybrid performance based on the genomic prediction of the lines. To do it 121 R parent lines were selected through molecular markers, crossed with 2 female lines, and their respective 188 hybrids were tested in field conditions for two years (2021 and 2022). The field test was conducted in an area with low phosphorus. The results obtained showed high accuracy in selecting non-tested hybrids.

Keywords: *Sorghum bicolor,* Accuracy, prediction ability, sorghum hybrids.

INTRODUCTION

In Brazil, more than 90% of sorghum is growing in areas located in the Cerrado biome, during the second harvest, from January to mid-March. The remaining vegetation of the Cerrado grows on old and weathered soils, which may be associated with high acidity. These soils are characterized by high aluminum saturation, low availability of nitrogen, phosphorus, potassium, calcium, magnesium, zinc, boron and copper, and reduced water retention capacity (Silva; Malavolta, 2000). Aluminum is one of the most abundant metals in the Earth's crust and, under acidic conditions, is released into the soil solution, taking on phytotoxic forms, such as Al^{3+} (Araki et al., 2000).

The presence of Al^{3+} in the soil can have a detrimental effect on plant growth and root development (Barros et al., 2020). It can inhibit root growth, resulting in shorter and thicker roots, with root tips being particularly affected by aluminum toxicity (Kochian et al., 2015). Soils with high levels of aluminum saturation, especially when associated with water deficit, can significantly reduce grain yield (Carvalho-Júnior et al., 2015). This could make cultivation areas unviable or considerably increase production costs. In addition, aluminum in the soil hinders the absorption of phosphorus, calcium, and magnesium and contributes to the adsorption of phosphorus in the soil (Echart and Cavalli-Molina, 2001).

In sorghum, the SbMATE gene encodes a transporter protein of the MATE (Multidrug and Toxic Compound Extrusion) family. This protein confers tolerance to aluminum by releasing citrate when activated by aluminum at root apices (Magalhães et al., 2007). However, when the AltSB locus (containing the SbMATE gene) was introduced into a sensitive line using marker-assisted introgression, the tolerance transfer to aluminum was incomplete compared to the tolerant donor parent (Jain et al., 2007). This incomplete transfer was associated with a decrease in SbMATE gene expression levels, indicating the involvement of regulatory factors in the control of its expression (Melo et al., 2013). In addition, single nucleotide polymorphisms

(SNPs) linked to aluminum tolerance and SbMATE gene expression have been identified in the region of approximately 51 to 54 Mbp on sorghum chromosome 9 (Jiang et al., 2015).

Genetic improvement allows us to increase productivity without the need to expand agricultural areas, which is beneficial both economically and environmentally. Therefore, this work aims to identify sorghum lines using gene-specific molecular markers for SbMATE and PSTOL 1. The present study aimed to select sorghum hybrids with competitive grain yield and the highest efficiency in phosphorus acquisitions under soils with low P availability through genomic prediction.

MATERIAL AND METHODS

A set of 480 $F_{2:5}$ progenies (Attachment 1) derived from crosses between two aluminum tolerance sources (SC549 and SC566-14) with 6 (six) elite lines of the sorghum breeding program (9503062, 9618158, 9910032, CMSXS180R, CMSXS182R and CMSXS110R) were genotyped with gene-specific markers to identify progenies containing favorable alleles of the SbMATE and SbPSTOL1 genes. The 480 progenies were genotyped with four SNPs associated with aluminum tolerance: AltSB_5519, AltSB_6083, AltSB_6083_CA and AltSB_8423 (Caniato et al., 2014). For phosphorus uptake efficiency, progenies were genotyped with four SNPs associated with increased phosphorus acquisition in SbPSTOL1 genes: Sb03g031680_1541pb, Sb03g006765_1998pb, Sb03g006765_2067pb, and Sb03g006765_2141pb (Hufnagel et al., 2014). After genotyping, 77 lines with favorable alleles from both genes were selected. Another 33 lines that did not present the markers, but with good characteristics for panicle size, flowering, plant height, and anthracnose tolerance, were added, completing 110 lines.

These 100 R (restorer) lines, were crossed with two A/B female lines (CMSXS 1006A and CMSXS 1008A). From this, 188 hybrids were generated, since some crosses were not successful. The 188 hybrids obtained were grown in a low-phosphorus area, with the check the cultivar BR007 and the line SC283. The BR007 cultivar is considered sensitive to aluminum and not efficient for phosphorus acquisition but responds with higher productivity when it comes to optimal conditions of phosphorus availability. On the other hand, the SC283 line is tolerant to Al^{3+} and efficient in phosphorus acquisition, being unresponsive to the increase of P in the soil, according to Matsumoto (2002). In addition, another 12 commercial hybrids from Embrapa and other companies (1G100, 1G233, 50A40, 50A60, DKB540, DKB550, DBK590, BRS373, BRS380, BRS330, BRS310, BRS3318) were used to evaluate their performance under low phosphorus conditions, forming a total of 202 genotypes evaluated.

Genotyping of the lines

Genotyping was done with the Kompetitive allele-specific PCR system (KASP, LGC Genomics http://www.lgcgroup.com/), as described in Hufnagel et al. (2014). The generation of markers for the SbMATE gene and SbPSTOL1 genes are described in Caniato et al. (2014) and Hufnagel et al. (2014), respectively.

Phenotyping of hybrids

Twelve commercial hybrids (BRS373, BRS380, BRS330, BRS310, BRS 3318, 1G100, 1G233, 50A40, 50A60, DKB540, DKB550, DKB590). To complete the list of genotypes tested, two checks were included: BR 007 and SC283. BR 007 is described as responsive to the addition of P in the soil, presenting better results than other lines when the soil conditions are optimal. The other check, SC283 described as efficient in uptaking phosphorus in deeper layers of the soil found by its root exploration (Bernardino et al., 2019). The phenotyping trials of the 200 hybrids, plus 2 lines were carried out in 2021 and 2022, during the off-season, at Embrapa Maize and Sorghum, in Sete Lagoas, 19°27'57" and 44°14'49"W, central region of Minas Gerais. The experimental plots consisted of 1 row of 3 m, with 0.5 m between rows, conserving ten plants per meter after thinning, and 3 reps. The lattice design, with additional controls in each block, and fixed effects for all variance components was used. The characteristics evaluated were: plant height, measured from the soil to the apex of the panicle; flowering, dated when 50% of the plants in the plot had open flowers up to the middle third of the panicle; grain yield, measured through the grain weight of the plot, and extrapolated to tons per hectare; thousand grains, by counting and weighing 1000 sorghum grains of each treatment.

Data analysis

Corrections of phenotypic data analysis were performed using the ASREML package, in R version 3.3.1, to obtain the adjusted variance components, *BLUEs* (*Best Linear Unbiased Estimation*) and *BLUPs* (*Best Linear Unbiased Prediction*), which were used in conjunction with genotypic data. For the subsequent analyses, the BLUEs were used.

First, the best unbiased linear estimators (BLUEs) for the upper crossover mean values of each hybrid were estimated as adjusted BLUEs averages for the testers effects. Next, the best genomic unbiased linear predictors (GBLUPs) for each hybrid were calculated using the genomic relationships of the additive genomic relationship matrix (A) in a general combining ability model. While it is possible to incorporate specific effects of combining ability into genomic prediction models (Kadam et al., 2016), this requires genomic information about the testers, which was not available in this study because some testers were commercial lines not available for genetic study.

$$y_{ijk} = \mu + E_i + T_j + G_k + \varepsilon_{ijk}$$

where y_{ijk} is grain yield of the hybrid k derived from the hybrid (Tester) j in environment i, μ is the overall average yield, E_i is the random effect of environment i, T_j is the random effect of line j, G_k is the fixed effect associated with the line or check hybrid k, and ε_{ijk} is the residual effect, which includes confounding effects of experimental error and interaction lines × environments.

The BLUEs for grain yield and weight of 1000 grains were used as input phenotypes for genomic prediction models in training sets and as genetic values observed in test sets to measure genomic prediction ability. In stage 2, the estimated breeding values for the hybrids were removed from the BLUEs datasets (Stage 1). Thus, the following model was suitable to obtain GBLUPs of grain yield values using the additive genomic relationship matrix (A):

$$\hat{Y}\,k.. = \mu + \hat{G}\,k + \varepsilon\,k$$

where the observed values $\hat{Y}\,k..$ are the estimated breeding values for hybrids grain yield or for the weight of 1000 grains obtained as BLUEs from the stage 1 analyses, μ is the overall mean, $\hat{G}\,k$ is the random effect of the line k modeled using the additive genomic relationship matrix (A) such that $G \sim N(0,\ A\sigma^2 G)$, and $\varepsilon\,k$ is the residual effect of the estimated breeding value. The GBLUPs in this model are predictions of breeding values of lines. Stage 2 analyses were performed with ASReml-R 4.0 (Sun et al., 2017).

Predictive ability was measured as the correlation between GBLUPs and BLUEs. For example, when the training set included the program lines, the pre-experiment j, G_k is the fixed effect associated with the line or the verification hybrid k and ε_{ijk} is the residual effect.

To assess the predictive ability three Cross Validation (CV) schemes were used. For all of them, 70% training population and 30% validation sets were applied. In the CV1 testcross hybrids were chosen randomly for validation. In CV2, R lines were randomly chosen, and each chosen R line had one of the two corresponding testcross hybrids randomly chosen for the validation. In CV3, the R lines were randomly chosen, and each of them had both of the corresponding testcross hybrids used for validation. The prediction accuracies of Grain Yield were also obtained by the comparison between the ability to accurately rank hybrids, allowing for discarding and advancement of hybrids based on predicted performance.

RESULTS AND DISCUSSION

Table 1 shows the joint results of the variance and heritability components for the hybrids evaluated in the field, under low phosphorus conditions. These results were obtained after correcting the means in two stages and obtaining the BLUEs and BLUPs. Subsequently, GBLUPS were calculated to obtain the additive genomic relationship matrices, based on the results of grain yield and 1000 grain weight. To adjust the genotype of the lines with the phenotype, the filter of markers was performed, and the Kinship matrix was generated. Next, cross-validation was performed and given the prediction results of the traits of lines and hybrids (Graph 1). The accuracy of the prediction was calculated as the correlation between the observed phenotype and the predicted one, divided by the square root of the trait's heritability (Yu et al., 2020).

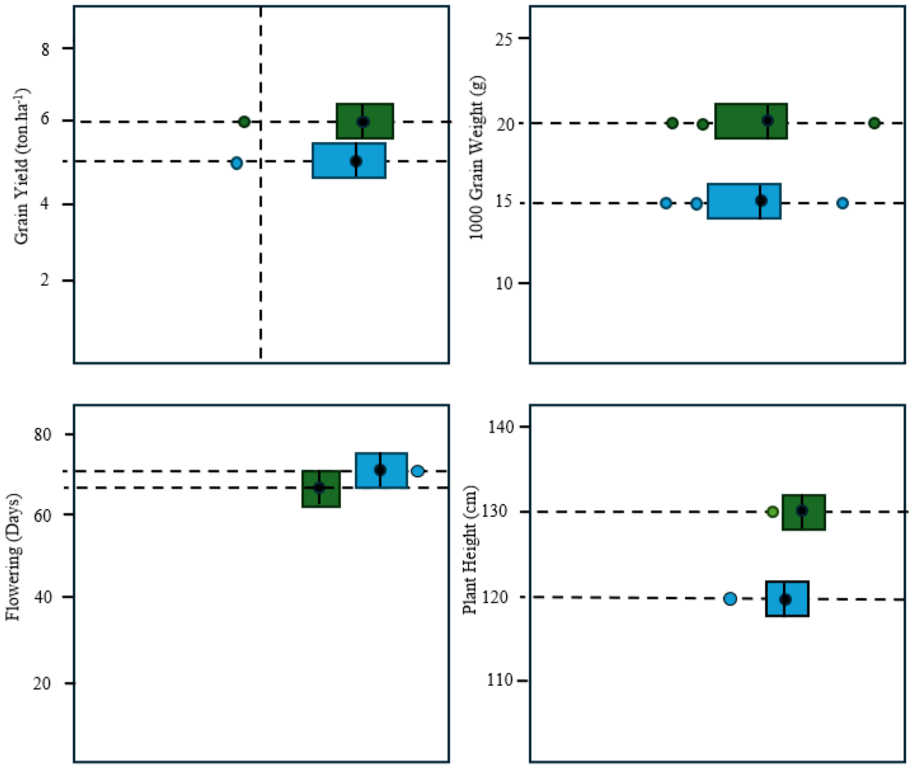
Table 1. Variance, heritability, and coefficient of variation components for traits evaluated in sorghum hybrids.

VS	**Significance**

Fixed effects[1]	Flowering Date (days)	Plant Height (cm)	Yield ($t.ha^{-1}$)	1000 Grain Weight (g)
R_K	***	ns	ns	ns
$Height_{ijklm}$	-	***	-	-
$Flowering_{ijklm}$	***	-	-	-
$Yield_{ijklm}$	-	-	***	-
$1000\ Grain\ Weight_{ijklm}$	-	-	-	***
ε_{ijklm}	-	-	-	-
Randon Effects[2]	**Component of variance**			
G_i	12.72	163.92	0.4461	5.16
$BL_l(k)$	0.01012	0.0471	0.0004595	0.0006482
$Plot_m$	7.99×10^{-8}	0.0002796	6.76×10^{-6}	2.27×10^{-8}
ε_{ijklm}	12.49	73.06	1.24	3.56
Minimum	67.00	85.00	0.367	7.95
Average	76.57	127.80	3.863	19.30
Maximum	90.00	220.00	10.60	30.34
CV%	4.64	10.02	16.80	12.48
Heritability	0.75	0.69	0.61	0.7186

*** Significance $p<0,001$ of probability Ns. Non-significant.

A range of 5.2 and 6.0 $ton.ha^{-1}$ Grain Yield was observed for the BLUEs, which is on average higher than the Brazilian average for sorghum grain. According to the CONAB (National Company of Agriculture and Supply) 2024/2025 grain sorghum production increased by 2.9% from the previous year and is projected to 4.42 $ton.ha^{-1}$ (Graphic 1). For the 1000-grain weight, the range was 15g to 20g. The Flowering date was between 70 and 75 days, fitting into the medium cycle hybrids range, and plant height was between 120 to 130 cm, considered standard for grain hybrids cultivars (Menezes et al., 2021). Nine hybrids were selected in the filtering and are listed in the table below (Table 2). Grain yield ranged from 3 ton ha^{-1} to 6.7 ton ha^{-1}. The highest grain yield was obtained by hybrid 113, with 6.7 ton ha^{-1}. However, the 50 hybrids, also included as a highlight for grain yield, were among the nine top 5%.



Graphic 1. Boxplot of best linear unbiased estimates (BLUEs) with the mean (Green box 2020 mean, Blue box 2021mean) for grain yield (ton.ha^{-1}), plant height (cm), 1000 Grain Weight (g) and Flowering (Days) for sorghum hybrids.

Table 2 presents the selection of 10% of the hybrids with the highest grain yield and the lowest ones, only considering the experimental hybrids.

Table 2. Selection of the 10% of the hybrids with the highest/lowest Grain Yield.

Highest yield	Grain Yield	Lowest yield	Grain Yield
G3	5.08	G1	3.50
G39	4.98	G17	3.46
G50	6.60	G27	3.39
G73	5.13	G30	3.34
G106	5.06	G46	3.26
G113	6.87	G48	3.41
G119	5.11	G55	3.26
G120	5.22	G56	3.41
G132	5.20	G58	3.45
G134	5.54	G61	3.31
G150	5.02	G63	3.30
G151	5.25	G69	3.27
G153	5.02	G75	3.18
G186	5.08	G84	3.47
G187	5.26	G91	2.64
G188	5.11	G112	3.26
G191	5.22	G117	3.48
G196	6.49	G144	3.42
G200	6.33	G161	3.33

The selection of 10% of the experimental hybrids was based on Grain Yield, and evidences the G113 with 6.87 ton.ha^{-1}. These hybrids are the ones selected and should be tested in more years in the non-optimal soil conditions to confirm their agronomic potential of P acquisition in soils with low availability of this nutrient. Also, the selection of 10% of the hybrids with the lowest grain yield was proceeded with the intent of recommending discarding some of the tested hybrids.

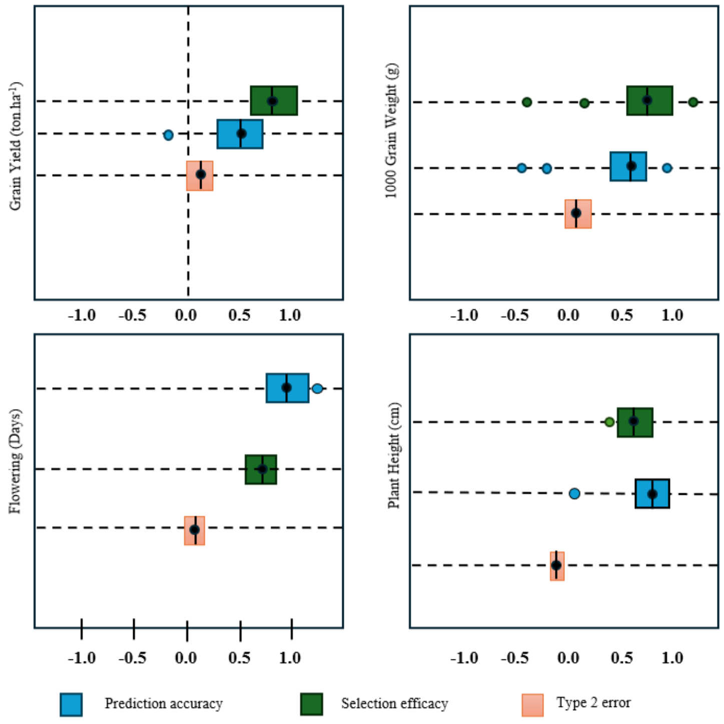
The mean prediction accuracy for Grain Yield for CV1 was 0.70; CV2 was 0.60 and CV3 was 0.75 (Figure 3). The prediction accuracy of CV1 and CV3 was higher than the CV2 scheme for Grain Yield and Flowering date. In 1000 Grain weight CV2 and CV3 were higher than CV1, and CV2 and CV3 were higher than CV1 for plant height.

Table 3. Cross-validation for grain sorghum characteristics.

Traits	Cross Validation schemes		
	CV1	CV2	CV3
Grain Yield	0.70	0.60	0.75
Flowering date	0.68	0.50	0.64
1000 Grain Weight	0.52	0.55	0.60
Plant height	0.56	0.60	0.60

None of the Cross-validation schemes obtained out of 5 repetitions had significant statistical differences. Although CV1 had the best prediction ability for Grain Yield and flowering date, two of the most practical characteristics were used to select genotypes. The scheme of Cros-validation is mentioned in other papers as the one associated with higher heritability, which is associated with a higher prediction of accuracy (Kent et al., 2023).

The CV3 prediction method demonstrated that the hybrid performance of an individual R line can effectively be predicted when data from related lines have been included in the model. The two populations (population of validation and population of training) of testcross hybrids exhibited differences in means, variances, and heritabilities for grain yield, flowering date, and plant height. Simulations indicate that the number of markers, heritability, and the count of genotypes influence prediction accuracy. The GBLUP method can achieve similar levels of prediction accuracy with fewer markers (Cerioli et al., 2022; Kent et al. 2023).



Graphic 2. Boxplots with the mean for prediction accuracy, selection efficacy, and type 2 error occurrence for Grain Yield, Flowering date, Plant Height, and 1000 Grain Weight.

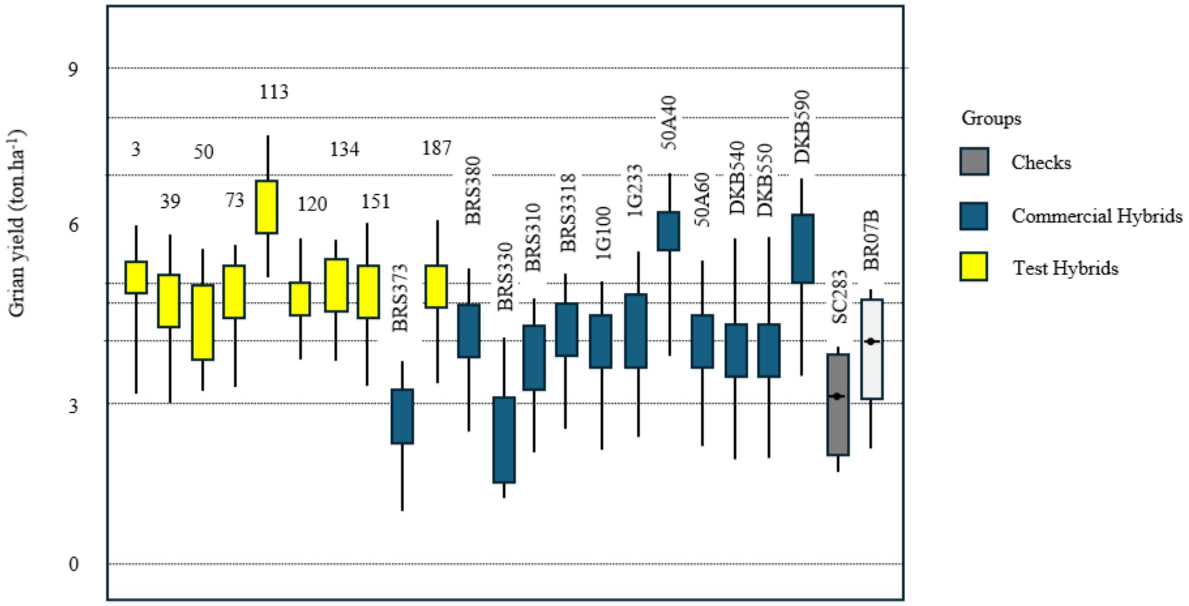
The predictive accuracy (Graphic 2) of the model used was expressive for the field characteristics, which presented, in general, results between 0.5 for 1000 grain weight and 0,89 for flowering date. For some other researchers, plant height showed high heritability also associated with high prediction ability (Hefner et al. 2011; Lipka et al. 2014; Burks et al. 2015). For the present study, the plant height prediction was -0.79 and the heritability 0.56.

Genomic prediction accuracy is usually assessed through the correlation between observed and predicted values. However, plant breeders prioritize accurately ranking line or hybrid performance to make decisions on which to keep or discard. This study could evaluate the ability to identify and eliminate low-yielding hybrids using genomic prediction data considering that the prediction efficacy was above 0.6 for all the characteristics in the study.

A selection intensity of 30% was established and was applied to Budhlakoti et al. (2022) when they tested sorghum hybrids in 8 environments with the lowest and highest prediction accuracies for both testcross hybrid populations ranging in between 0.60 and 0.71. In this case, it was possible to increase the capacity of selection based on predicted performance classified individual inbred lines into the corresponding phenotypic classes, to select and discard with more efficiency. In the present study, the selection index was lower because the volume of data involved in the analyses was shorter than the one above-referenced.

According to Kent et al. (2023) Type 2 errors are unavoidable in genomic prediction. However, if high-quality training data is used, genomic prediction is not influenced by spatial effects that could obscure results and increase the likelihood of type 2 errors, which is often seen in yield trials. While it is impossible to eliminate type 2 errors in plant breeding, understanding where these errors arise can help reduce their occurrence.

In general, the experimental hybrids presented interesting results, since they were under stress conditions, in an area of low phosphorus, and still obtained grain yield results above 5.0 ton ha^{-1}. It is worth highlighting the grain yield of hybrid 113, which resulted in 6.7 ton ha^{-1}. The commercial hybrids had more modest results compared to the hybrids, with the best being 50A40 and DKB590 with 6.3 ton ha^{-1}. BR007 is considered non-responsive to P, although the 3.7 ton.ha^{-1} yield was a little higher than the SC283 (3.0 ton.ha^{-1}), which usually has a better phosphorus acquisition under soils with low P availability (Graphic 3). In this case the trigger to the roots exploring the available nutrients, like P, in deeper layers of the soil is the scarcity of the element. phosphorus.



Graphic 3. Grain sorghum hybrids selected for grain yield (5% index), commercial hybrids, and check cultivars SC283 and BR007.

The performance of grain yield in low-phosphorus (low-P) conditions has been documented as a trait with high heritability and minimal genotype by environment interaction. This is evidenced by a strong genetic correlation between grain yield under control conditions and low-P conditions. However, crossover interactions can occasionally occur, especially in high-yielding genotypes (Bernardino et al., 2019; Hufganel et al., 2024). The prediction accuracy normally increases proportionally with heritability, as shown in studies conducted by Combs and Bernardo (2013) and Lehermeier et al. (2014). Fernandes et al. (2018) showed that the accuracy increases with higher heritability in sorghum, wheat, and corn, considering root characteristics. With this study it was possible to observe a prediction ability of around -0.4, which shows the ability to select based on genomic prediction models. For a sorghum breeder, the grain yield is the most important trait (Graphic 3) and the selection of traits based on statistical models that well correlate early season characteristics with grain yield can give to the breeder years of advantage with a faster selection (Silva et al., 2022).

CONCLUSION

Genomic prediction has promising results that can be adhered to the sorghum breeding program, to select hybrids with high yield performance in environments with low P availability. The genomic prediction has also the potential of and discard identify hybrids to discard, once they are not tolerant or responsive to adverse conditions. The genomic prediction for grain yield showed accuracy higher than 0.6 for Grain Yield, Plant Height, 1000 Grain Weight, and Flowering date. Also, the efficacy of prediction expressed promising results, especially for Grain Yield, indicating an accuracy of 0.67 and predicted values were close to the data collected in the field.

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CHAPTER 2

GENOMIC PREDICTION FOR ROOT MORPHOLOGY IN SORGHUM LINES UNDER LOW PHOSPHORUS CONDITION.

ABSTRACT

The abiotic stresses caused by Al toxicity and low phosphorus availability have the same chemical properties, being cause and consequence of each other. In other words, Al molecules are hydrolyzed to the ionic form Al^{3+} under low phosphorus availability. This ionic form of Al can be assimilated by the roots, causing permanent damage and reducing crop yield. In addition, in soils with low P, phosphorus fixation occurs with oxides of Al, Fe, and soil clay. These soil components change the P of the orthophosphate ($H2PO4^-$ and HPO42-) to the unavailable form that restricts root uptake. One of the strategies to overcome this situation has been research for crops like sorghum, which have greater efficiency in phosphorus acquisition and can significantly reduce the demand for fertilization. This paper aimed to use genomic prediction tools to test the accuracy of prediction in root traits for lines tested in low-phosphorus hydroponics systems. Genomic prediction can be an effective method to predict the performance of the lines when they were not tested, and to predict combinations without extensive field testing.

Keywords: Breeding values, P uptake, *Sorghum bicolor*.

INTRODUCTION

Sorghum is considered a resilient crop because it can grow where most cereals cannot thrive due to its high tolerance to heat and drought (Sapkota et al., 2020; Borrel et al., 2014). According to Morris et al. (2013), this adaptability is due to sorghum's genetic and physiological diversity. However, one of the main challenges sorghum producers is the presence of acidic soils with high concentrations of aluminum, which leads to low phosphorus availability (Menezes et al., 2016).

Most Brazilian soils are acidic, especially those found in Cerrado regions (Quaggio, 2000). According to Too et al. (2020), high concentrations of aluminum in the soil can reduce grain yield and green mass in sorghum by up to 40%. Aluminum can cause significant damage to plants, including inhibiting root growth, leading to reduced water and nutrient uptake (Santos et al., 2018).

A gene known as PHOSPHORUS STARVATION TOLERANCE 1 (PSTOL1), identified by Gamuyao et al. (2012), is the first gene associated with P efficiency (tolerance to low soil P) in rice. This gene encodes a receptor-like kinase and is responsible for a crucial quantitative trait locus for P uptake in rice root (Wissuwa et al., 2005). In sorghum, the improvement of phosphorus acquisition efficiency can lead to a yield increase of up to 0.6 t ha^{-1}. Sorghum lines that overexpress PSTOL1 demonstrated increased total root length and root surface area as well as higher P uptake and grain yield under low P conditions compared to the control (Bernardino et al., 2020).

PSTOL1 is expressed in the primordia of the crown root and in the parenchyma, cells located outside the peripheral vascular cylinder, where the crown roots form in rice (Huang et al., 2014). In sorghum, several homologs of OsPSTOL1 have been identified through association mapping using candidate genes associated with P efficiency in the field (grain yield and P uptake in low-P soil) and/or in the laboratory (changes in topology and root growth and

P uptake) (Hufnagel et al., 2014). In this study by Bernardino et al. (2019), sorghum SbPSTOL1 genes appear to alter the morphology and architecture of the root system, leading to increased grain yields in field studies in Brazilian soil with low P content (Bucksch et al., 2014). These data suggest a consistent effect of the target alleles on different sorghum environments and genetic origins (Too et al., 2020; Bernardino et al., 2019).

Plants deficient in phosphorus (P) show a reduction in leaf length, leaf area and number of leaves. In field experiments, several characteristics can be evaluated, such as flowering, plant height, grain yield, P content, and dry mass in grains and straw (Bernardino et al., 2019). Changes in root system architecture, such as longer and thinner lateral roots in the topsoil, are crucial for plants to effectively absorb P from the soil (Lynch, 2011). However, few genes have been validated for their effect on the root system and crop adaptation in soils with low P content.

The present study uses genomic selection root traits correlated to grain yield to assess the performance of a group of grain sorghum lines in low-phosphorus hydroponics systems.

MATERIAL AND METHODS

Genetic Material

Aluminum phenotyping methods

One hundred lines were selected through molecular markers and, phenotypic evaluated in nutrient solution for Al as described in Caniato et al. (2014) and for efficiency in the use of P as described by Hufnagel et al., 2014). Phenotyping for Al tolerance was based on the inhibition of root growth caused by Al in nutrient solution (Caniato et al., 2014), whose protocol is routinely used in our laboratories. In summary, seminal root growth inhibition were evaluated in nutrient solution containing 27 μM Al^{3+} compared to a control without Al^{3+}, both in nutrient solution with pH 4.0. The evaluations were made 5 days after the imposition of Al stress. The characteristics collected were root growth (mm) of 1-3 days, 3-5 days and 5-7 days and the ratio (%) between the net length of the seminal root in the presence and absence of Al.

To evaluate the efficiency in the use of P, three uniform seedlings were used for each replication, and placed in a modified Magnavaca nutrient solution (pH 5.65) (Magnavaca et al., 1987) with 2.5 μM P (low P) in a system composed of file folders lined with germination paper (Hund et al., 2009). After 13 days, the root system was photographed with a digital camera (Nikon D300S SLR). The images obtained were analyzed using the RootReader2D and WinRhizo v. 4.0 software (Regént Systems, Quebec, Canada). The measured characteristics: length (cm), average diameter (mm), total surface area (cm²), root surface area with diameter between 0-1 mm, The experiment was conducted in a growth chamber with an average daytime temperature of 27 ± 3 °C, a night temperature of 20 ± 3 °C and a photoperiod of 12 hours, under continuous aeration and the nutrient solution changed every three days (Sousa et al., 2012).

Phosphorus phenotyping methods

The experiment was conducted in pastes containing germination paper and Hoagland's nutrient solution was modified with pH adjusted to 5.65 and changed every three days, according to Liu et al. (1998). Sorghum genotypes were evaluated in solution with phosphorus concentrations of 0; 2,5; 150 and 250 M in a growth chamber with a temperature of 25 °C and a photoperiod of 12 hours. The seedlings were analyzed 3, 6, 9, 12 and 15 days after transferred to the pastes. The experimental design was completely randomized, with each treatment represented by three plants.

Association analysis and genomic prediction

The lines were previously selected and genotyped with 4 SNPs associated with aluminum tolerance: AltSB_5519, AltSB_6083, AltSB_6083_CA and AltSB_8423 (Caniato et al., 2014). For phosphorus uptake efficiency, progenies were genotyped with 4 SNPs associated with increased phosphorus acquisition in SbPSTOL1 genes: Sb03g031680_1541pb, Sb03g006765_1998pb, Sb03g006765_2067pb, and Sb03g006765_2141pb (Hufnagel et al., 2014).

Genotyping was done with the Kompetitive allele-specific PCR system (KASP, LGC Genomics http://www.lgcgroup.com/), as described in Hufnagel et al. (2014). The generation of markers for the SbMATE gene and SbPSTOL1 genes are described in Caniato et al. (2014) and Hufnagel et al. (2014), respectively.

An estimation of the effect of the markers was used on the characteristics associated with Al tolerance and P acquisition, and the genotypic and phenotypic data from the 100 lines were used. The observed estimates of –log10(P) were compared with the expected values of –log10(P), through a Q-QPlot graph, and the one with the best fit was selected, using the relationship matrix as described in Hufnagel et al. (2014). After choosing the most appropriate model, SNP markers with a P-value lower than the pre-established threshold were considered significant. Manhattan-Plot plots were used to show the physical positions of the markers, plotted along the axis of the abscissas (X) and the – log10(P) plotted on the axis of the ordinates (Y).

The analyses were done in the TASSEL v 5.0 program (Bradbury et al., 2007) and R (R Team, 2018). These marker effects were applied to predict the breeding values of the 100 sorghum lines for each trait. To evaluate the prediction accuracy, the predicted breeding values based on the effects of gene-specific markers were compared with the predicted breeding values based only on the phenotypic data obtained in the evaluations of the lines in the nutrient solution.

The prediction of the breeding values was important to evaluate the use of the effects of gene-specific markers to predict the performance of lines in terms of Al tolerance and efficiency in the use of P in Embrapa's sorghum breeding program. Based on the models selected above and predicted breeding values for the lines, genomic predictions of the lines were performed.

RESULTS AND DISCUSSION

Plant breeding programs have studied genes that express plant efficiency in phosphorus acquisition (sbPSTOL1) and aluminum tolerance (AltSB) in sorghum. The efficiency of phosphorus use is associated with the ability of these plants to explore the soil, modulations of P transporters, exudation of organic acids, and changes in the architecture of the root system. This is essentially done by increasing the root area and the number of fine roots. Molecular studies have been increasing the gain of time in selection when associated with genomic selection and genomic prediction. Studies have validated sorghum genes encoding PSTOL1 proteins through associative mapping and QTL mapping (Hufnagel et al., 2014; Bernardino et al., 2019). The results of the association mapping analyses indicate a correlation between the genes Sb07g002840, Sb03g031700, Sb03g031690, Sb03g031680, Sb03g031670, Sb03g006765 and traits of root morphology, P acquisition, and grain yield in tropical soils with low P availability. Specifically, the Sb07g002840 gene co-located with QTLs for root diameter and grain yield.

In Figure 1, on the Manhattan plot was possible to identify genomic regions that are associated with yield, that is the interest trait. One significant SNP (S3_71178053) was found for relative root length, which explained 24% of the genotypic variance and had a notable impact on length (Figure 1). In the work of Leiser et al., (2014) three SNPs that were closely associated with S3_71178053 were identified, with strong associations to grain yield, observed on chromosomes 6 and 7.

Nine SNPs in gene Sb07g023120 and one SNP (S7_57976035) in gene Sb07g023130 on chromosome 7 showed a strong association ($-\log10(p) > 4.5$). None of the highly associated

SNPs on chromosome 6 were located within coding regions. The SNP S3_71178053 was the most significant for root length under both control and low P conditions. Chromosome 10 exhibited a region highly associated with grain yield under P. Additionally, a single SNP on chromosome 5 and a second SNP on chromosome 1 caused a missense variant in gene Sb01g032090 (Figure 1).

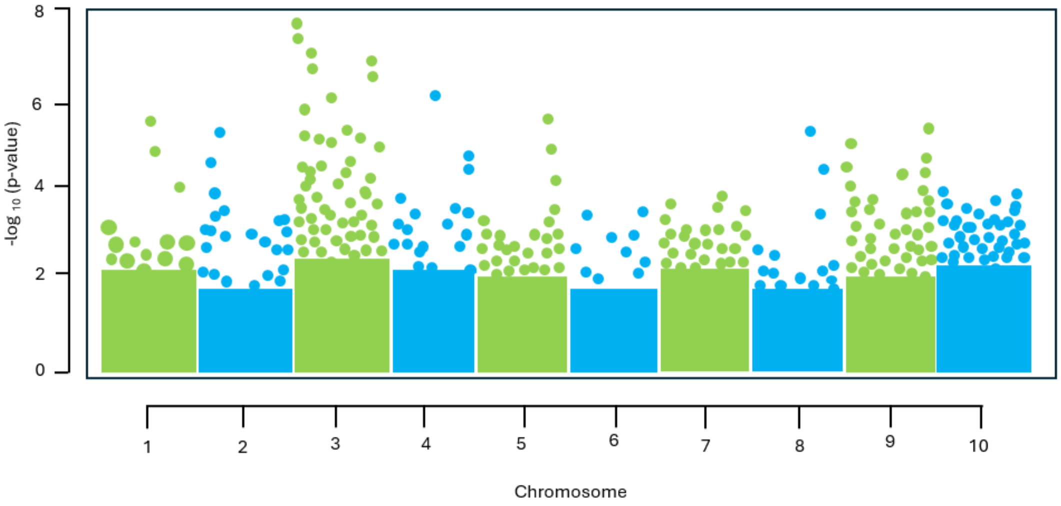

Figure 1. Manhattan plot for sorghum root traits BLUPs under control and low phosphorus of 100 lines genotyped with 167 K SNPs with p values as log10 scale and Bonferroni threshold at p<0.05.

These marker effects were used to predict the breeding values of the 100 sorghum lines for each trait. To evaluate the prediction accuracy, the expected breeding values based on the effects of gene-specific markers were compared with the predicted breeding values based only on the phenotypic data obtained from the lines in the nutrient solution. This strategy was important in estimating the effects of gene-specific markers to predict the performance of lines in terms of Al tolerance and efficiency in uses of P. Based on the models selected above and predicted breeding values for the lines, genomic predictions of the line's performance.

According to Bernardino, there is a high correlation between phosphorus acquisition efficiency, phosphorus use efficiency, and grain yield (r = 0.85 and 0.97). This emphasizes the importance of phosphorus acquisition for phosphorus use efficiency. Previous researchers like Lopéz-Aredondo et al. (2014) and Lynch (2011) has explored the relationship between root morphology traits under low phosphorus availability through genetic correlation analysis. The present study found that a decrease in root diameter was generally linked to an increase in total root surface area (r = -0.77) (Figure 2). This suggests that there are genetic factors promoting the development of finer roots to increase root surface area.

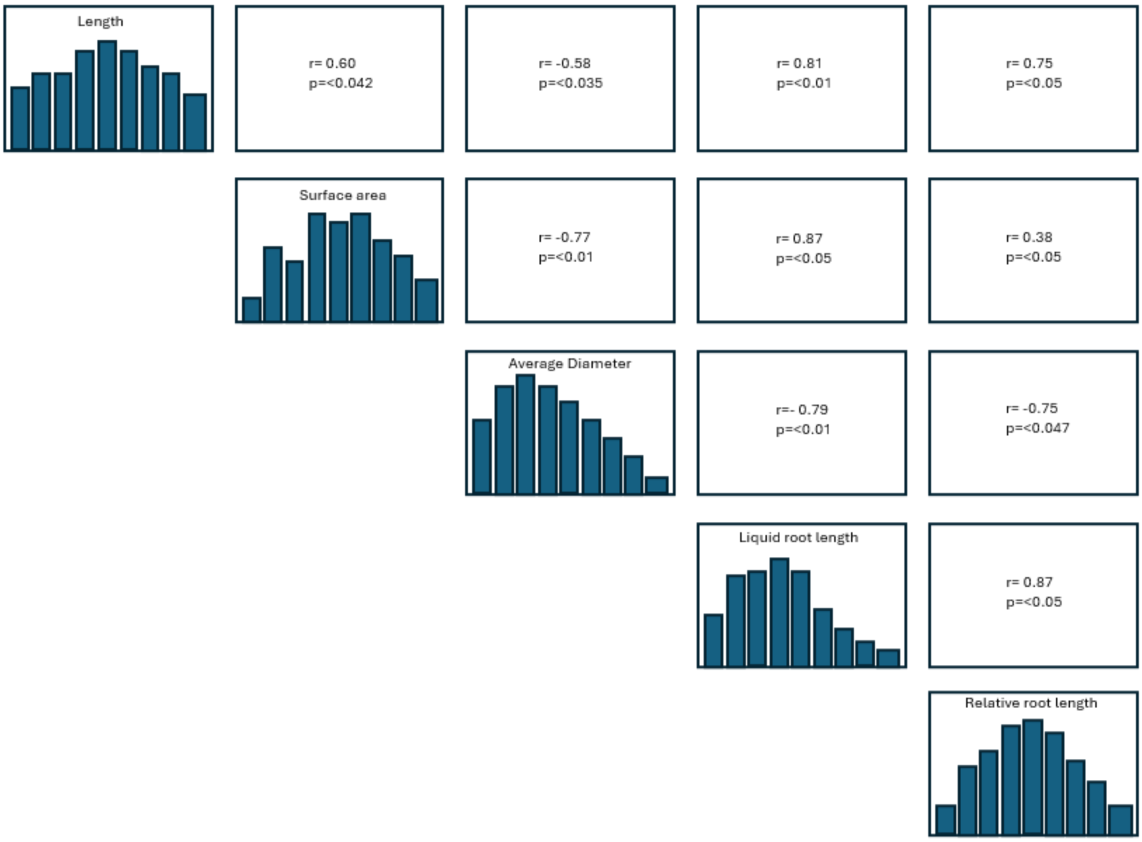

Figure 2. Genetic correlation analysis of root morphology traits. Pearson correlation coefficients (r) and p-values (p) are shown.

The effectiveness of genomic prediction is typically evaluated by comparing the observed and predicted values. In this study, the accuracy of the prediction was higher than 0.48

for all the root traits. For practical purposes, these results can be used to select lines that have not been tested. The breeding values can be applied not only for the selection of potential parent lines but also to discard lines with low yields.

Table 1. Prediction ability (correlations between observed line mean values and genomic prediction values) for Root Length, Superficial Area, Average Diameter, Liquid Diameter, Liquid Length of Seminal Root, and Relative Length of Seminal Root.

Root trait	Prediction ability (r)	Accuracy (h^2)
Root length (mm)	0.55	0.48
Superficial Area (mm)	-0.30	0.56
Average Diameter (mm)	-0.45	0.59
Liquid Length of Seminal Root (mm)	0.50	0.71
Relative Length of Seminal Root (%)	0.35	0.72

Some of the lines selected (Table 2) by molecular markers were also the best for the root traits Liquid length of Seminal Root (LLSR) and Relative length of Seminal Root (RLSR). The molecular marker selection was presented in the previous chapter in a descriptive analysis format. For this study, the actual root observations are presented to show the match between phenotypic data and the molecular marker selection. In the table below we can find some of these results that converge with Bernardino et al. (2016) and Santos et al. (2018) average founds for the root trait in the case. The significance is observed, and the best lines selected have between 61.02% (G081) and 82.15% (1909G085) of relative length (RLSR). Changes in root structure such as increased root length can improve phosphorus uptake and grain yield in low-phosphorus soils, according to Bernardino et al. (2019). The line 1909G085 had more RLSR and LLSR, and in Gladman et al. (2022), the authors support the fact that very fine roots correlated with a higher surface area of both fine and thicker roots, and it lead to higher grain yield.

Table 2. Lines selected via molecular markers for AltSB and PSTOL 1, and results for Liquid Length of Seminal Root (LLSR), mm, and Relative Length of Seminal Root (RLSR), %.

Lines Code	R lines	LLSR	RLSR
1909G083	CMSXS180R * BR012(SC566-14)-58-1-2-1	50.61**	71.20**
1909G085	CMSXS182R * BR012(SC549)-4-1-1-1	61.59**	82.15**
1909G045	9910032 * BR012(SC549)-1-1-1-1-1	56.63**	69.78**
1909G010	((BR012RxCMSXS225)2xMR732)-01-4-1-1-1	64.35**	81.21**
1909G063	9503062 * BR012(SC549)-1-1-1-1	72.60**	64.50**
G081	((BR012RxCMSXS225)2xMR732)-06-5-1-5-1	49.03**	61.02**
G080	((BR012RxCMSXS225)2xMR732)-02-7-1-4-1	58.92**	80.00**
1909G053	9618158 * BR012(SC566-14)-100-1-1-1-1	57.58**	76.00**
SC283		74.35**	81.20**
BR007B		21.65**	31.24**

CONCLUSIONS

This study shows that genomic prediction tools can be used to predict breeding values of sorghum lines evaluated under low P conditions, showing acceptable accuracy for selection. Genomic prediction can be an effective method to predict the performance of the lines when they were not tested, and also to predict combinations without extensive field testing.

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Appendix 1 – Supplemental table with the Pedigree of the lines evaluated for root traits.

Trat	Pedigree	Trat	Pedigree	Trat	Pedigree
1	TX430R * BR012(SC566-14)-5-1-2-1	45	CMSXS182R * BR012(SC549)-118-2-1-1	89	9503062 * BR012(SC549)-66-1-1-1
2	CMSXS180R * BR012(SC549)-9-2-1-1	46	CMSXS182R * BR012(SC549)-105-1-1-1	90	9503062 * BR012(SC549)-147-1-3-1
3	CMSXS180R * BR012(SC549)-8-1-1-1	47	CMSXS182R * BR012(SC549)-105-1-2-1	91	TX430R * BR012(SC566-14)-74-3-1-1
4	CMSXS180R * BR012(SC549)-5-1-1-1	48	TX430R * BR012(SC549)-26-1-1-1	92	TX430R * BR012(SC566-14)-108-2-1-1
5	9910032 * BR012(SC549)-1-1-1-1	49	TX430R * BR012(SC549)-50-2-1-1	93	TX430R * BR012(SC566-14)-121-1-1-1
6	CMSXS182R * BR012(SC549)-4-1-1-1	50	TX430R * BR012(SC549)-36-1-1-1	94	CMSXS180R * BR012(SC566-14)-14-1-2-1
7	CMSXS182R * BR012(SC549)-4-1-2-1	51	TX430R * BR012(SC549)-42-1-1-1	95	9910032 * BR012(SC549)-13-1-1-1
8	CMSXS182R * BR012(SC549)-2-1-1-1	52	TX430R * BR012(SC549)-77-1-1-1	96	TX430R * BR012(SC566-14)-8-2-1
9	9503062 * BR012(SC566-14)-6-1-3-1	53	TX430R * BR012(SC549)-113-1-1-1	97	CMSXS180R * BR012(SC566-14)-60-1-1
10	9910032 * BR012(SC566-14)-1-1-1-1	54	TX430R * BR012(SC549)-91-1-1-1	98	CMSXS180R * BR012(SC549)-26-1-1
11	CMSXS182R * BR012(SC566-14)-8-1-1-1	55	TX430R * BR012(SC549)-91-1-2-1	99	(BR012RxCMSXS225)2
12	9910032 * BR012(SC549)-3-1-1-1	56	TX430R * BR012(SC549)-87-2-1-1	100	BR012(SC549)
13	9503062 * BR012(SC566-14)-2-1-1-1	57	9503062 * BR012(SC566-14)-57-1-2-1	101	BR012(SC566-14)
14	9503062 * BR012(SC566-14)-8-1-1-1	58	9503062 * BR012(SC566-14)-57-1-3-1	102	CMSXS180R
15	9503062 * BR012(SC566-14)-7-2-1-1	59	9910032 * BR012(SC566-14)-9-1-1-1	103	CMSXS 182R
16	9503062 * BR012(SC566-14)-7-2-2-1	60	9910032 * BR012(SC566-14)-7-1-1-1	104	MR732
17	9503062 * BR012(SC566-14)-7-2-3-1	61	9910032 * BR012(SC566-14)-111-1-1-1	105	TX430R
18	9910032 * BR012(SC549)-4-4-1-1	62	9910032 * BR012(SC566-14)-93-1-1-1	106	9503062
19	CMSXS180R * BR012(SC549)-4-1-2-1	63	9910032 * BR012(SC566-14)-187-2-1-1	107	9618158
20	CMSXS180R * BR012(SC549)-4-1-3-1	64	CMSXS182R * BR012(SC566-14)-15-1-1-1	108	9910032
21	9618158 * BR012(SC549)-2-1-1-1	65	CMSXS182R * BR012(SC566-14)-32-1-1-1	109	ATF13B
22	9618158 * BR012(SC566-14)-9-1-1-1	66	CMSXS182R * BR012(SC566-14)-86-1-1-1	110	ATF14B
23	9910032 * BR012(SC549)-4-1-1-1	67	9618158 * BR012(SC566-14)-36-1-1-1		
24	9503062 * BR012(SC549)-1-1-1-1	68	9618158 * BR012(SC566-14)-71-2-2-1		
25	9910032 * BR012(SC549)-4-2-1-1	69	9618158 * BR012(SC566-14)-60-1-1-1		
26	CMSXS182R * BR012(SC549)-4-1-1-1	70	9618158 * BR012(SC566-14)-84-1-1-1		

27	9618158 * BR012(SC549)-19-2-1-1	71	9618158 * BR012(SC566-14)-100-1-1-1
28	9618158 * BR012(SC549)-43-2-1-1	72	CMSXS180R * BR012(SC549)-5-1-2
29	TX430R * BR012(SC566-14)-37-1-1-1	73	9618158 * BR012(SC549)-25-1-1
30	TX430R * BR012(SC566-14)-74-3-2-1	74	9618158 * BR012(SC549)-93-1-1
31	TX430R * BR012(SC566-14)-132-2-1-1	75	CMSXS180R * BR012(SC566-14)-100-1-1
32	TX430R * BR012(SC566-14)-132-3-1-1	76	CMSXS180R * BR012(SC549)-7-1-1
33	TX430R * BR012(SC566-14)-132-3-2-1	77	CMSXS180R * BR012(SC549)-86-1-2
34	CMSXS180R * BR012(SC566-14)-57-1-1-1	78	((BR012RxCMSXS225)2xMR732)-01- 4
35	CMSXS180R * BR012(SC566-14)-47-1-1-1	79	((BR012RxCMSXS225)2xMR732)-02- 4
36	CMSXS180R * BR012(SC566-14)-64-1-2-1	80	((BR012RxCMSXS225)2xMR732)-02- 7
37	CMSXS180R * BR012(SC566-14)-103-2-1-1	81	((BR012RxCMSXS225)2xMR732)-06- 5
38	CMSXS180R * BR012(SC566-14)-103-3-1-1	82	((BR012RxCMSXS225)2xMR732)-07- 4
39	CMSXS180R * BR012(SC549)-76-1-1-1	83	((BR012RxCMSXS225)2xMR732)-08- 5
40	9910032 * BR012(SC549)-36-2-1-1	84	9618158 * BR012(SC549)-1-1-2-1
41	CMSXS182R * BR012(SC549)-41-1-1-1	85	TX430R * BR012(SC549)-1-1-1-1
42	CMSXS182R * BR012(SC549)-56-1-1-1	86	9910032 * BR012(SC566-14)-2-3-1-1
43	CMSXS182R * BR012(SC549)-118-1-2-1	87	9618158 * BR012(SC566-14)-3-1-2-1
44	CMSXS182R * BR012(SC549)-131-1-1-1	88	(BRP13R C-4)-8-3-1-1-1