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ASSOCIATIVE MAPPING FOR EXOTIC SOYBEAN GERMPLASM GRAIN YIELD IN HIGH TEMPERATURES[1]

CAMILA CAMPÊLO DE SOUSA[2], UBIRAJARA SANTANA ASSUNÇÃO[3], MÔNICA CHRISTINA FERREIRA[4], ÂNGELA CELIS DE ALMEIDA LOPES[5], REGINA LÚCIA FERREIRA DOS SANTOS[5], JOSÉ BALDIN PINHEIRO[6]*

ABSTRACT - Soybeans are among the world's main crops because they are excellent sources of proteins, micronutrients, and oil. Considering that abiotic stress affects agribusiness, resulting in losses, the grain yield of the crop must be maintained even at high temperatures. In this context, the objective of this study was to select markers related to soybean yield assessed under high temperatures, using associative mapping. The mapping population included 80 soybean PIs and 15 controls. For phenotyping, genotypes were evaluated at high temperatures in an experiment conducted in Teresina (in the state of Piauí) and four characters of interest for agronomy were evaluated: height of the plant when mature, agronomic value, 100-seed weight, and grain yield. Genotyping was carried out using the Affymetrix Platform (180 K Axiom® Soybean Genotyping Array), and the imbalance in the connection between pairs of markers was calculated through the coefficient of determination using the fast permutation test. The analysis of the association between markers and the phenotype of interest was carried out using a generalized linear model approach, including phenotyping data, SNP markers, and information on population structure. The results revealed that 34.06% of loci showed a significant linkage disequilibrium ($p < 0.001$), and 16 significant associations were found for the four characters related to heat tolerance. These associations can aid breeders that aim to incorporate high temperature tolerance in programs of soybean genetic improvement via selection assisted by markers.

Keywords: *Glycine max*. Linkage disequilibrium. Marker assisted selection.

MAPEAMENTO ASSOCIATIVO PARA PRODUTIVIDADE DE GRÃOS EM GERMPLASMA EXÓTICO DE SOJA SOB ALTAS TEMPERATURAS

ABSTRACT - A soja está entre as principais culturas do mundo, pois é uma excelente fonte de proteínas, micronutrientes e óleo. Considerando que o estresse abiótico afeta o agronegócio, acarretando em perdas, a produtividade de grãos da cultura deve ser mantida mesmo sob altas temperaturas. Nesse contexto, o objetivo deste trabalho foi selecionar marcadores relacionados à produtividade da soja, avaliada em altas temperaturas, por meio de mapeamento associativo. A população de mapeamento incluiu 80 PIs de soja e 15 controle. Para a fenotipagem, os genótipos foram avaliados em altas temperaturas em um experimento realizado em Teresina (estado do Piauí) e foram avaliados quatro caracteres de interesse agronômico: altura da planta na maturidade, valor agronômico, peso de 100 sementes e rendimento de grãos. A genotipagem foi realizada por meio da Affymetrix Platform (180 K Axiom® Soybean Genotyping Array) e o desequilíbrio de ligação entre pares de marcadores foi calculado por meio do coeficiente de determinação, por meio do teste de permutação rápida. A análise da associação entre os marcadores e o fenótipo de interesse foi realizada por meio de uma abordagem de modelo linear generalizado, incluindo dados de fenotipagem, marcadores SNP e informações sobre a estrutura populacional. Os resultados revelaram que 34,06% dos loci apresentaram desequilíbrio de ligação significativo ($p < 0{,}001$), sendo encontradas 16 associações significativas para os quatro caracteres relacionados à tolerância ao calor. Essas associações podem auxiliar os melhoristas que buscam incorporar a tolerância a altas temperaturas em programas de melhoramento genético da soja via seleção assistida por marcadores.

Keywords: *Glycine max*. Desequilíbrio de ligação. Seleção assistida por marcadores.

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*Corresponding author

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[2] Department of Sciences of Codó, Universidade Federal do Maranhão, Codó, MA, Brazil; camila.campelo@ufma.br – ORCID: 0000-0002-1403-2447.

[3] Departament of Distance Education, Universidade Federal do Piauí, Teresina, PI, Brazil; birabiologia@hotmail.com - ORCID: 0000-0003-1402-353X.

[4] Department of Germplasm Development, Syngenta, Uberlândia, MG, Brazil; monicachrisferreira@gmail.com – ORCID: 0000-0001-6166-0174.

[5] Department of Plant Sciences, Universidade Federal do Piauí, Teresina, PI, Brazil; acalopes@ufpi.edu.br – ORCID: 0000-0002-9546-5403, rlfgomes@ufpi.edu.br – ORCID: 0000-0002-7700-6959.

[6] Department of Genetics, Luiz de Queiroz College of Agriculture, Universidade de São Paulo, Piracicaba, SP, Brazil, jbaldin@usp.br – ORCID: 0000-0003-1294-2081.

INTRODUCTION

Soybeans are among the world’s main commodities due to their many uses in both the food and biofuel markets; not to mention that soybean oil, in addition to essential micronutrients and vitamins, has excellent culinary properties beneficial to health (GU; WU, 2013; POLETTO, 2019; RUDDLE et al., 2013).

Although there is a large worldwide grain yield, abiotic stresses cause large losses in soybean yield (BARBOSA et al., 2012). Abiotic stresses are the main cause of soybean production decline, which occurred in Brazil in the harvest of 2019/2020, with an estimated loss of 5.554 million tons (HIRAKURI, 2021). Considering that global climate changes are already affecting agriculture, there is a clear need for productive crops that can withstand abiotic stresses, such as high temperatures. Increases above 1 °C can restrict the growth of many crops, including soybeans (PELLEGRINO; ASSAD; MARIN, 2007). As a result, it is necessary to identify and select soybean genotypes that are resistant to high temperatures in different regions.

Considering that cultivated soybeans have a narrow genetic base, wild soybeans that are generally not adapted are used to search for agronomically interesting characters. The plant introduction (PIs) genotypes have been phenotyped and genotyped for use in improvement programs (QIU et al., 2011), but to use these genotypes, precise methods of phenotyping, numerous functional markers, high-performance genotyping, and efficient improvement programs are essential.

According to Bita and Gerards (2013), genetic improvement in tolerance to high temperatures can be made following current conventions that is, by subjecting the genotypes to high temperature conditions and selecting the most productive ones, or by using molecular strategies, the most common of which are transgenics and assisted selection via molecular markers. One of the tools used by breeders to do so is associative mapping, which is an analytical approach used in the search for Quantitative Trait Loci (QTLs) related to characters of interest, which can be used during improvement by selection assisted by markers (ZIEMS et al., 2014). The basic principle of associative mapping is the breakdown of a sample of a population with no known kinship into homogeneous sub-populations (GUPTA; RUSTGI; KULWAL, 2005). Previous studies have carried out associative mapping of soybeans to detect loci associated with different characters of interest, such as resistance to disease (KANG et al., 2012), size and shape of the seeds (NIU et al., 2013), chlorosis due to iron deficiency (LAMKEY; HELMS; GOOS, 2013), tolerance to aluminum (KORIR et al., 2013), oil and protein contents (PRIOLLI et al., 2019), interactions between the growth time and environmental conditions (MAO et al., 2017), and grain yield (HU et al., 2014). This study is a pioneer in the realization of associative mapping for analyzing tolerance of soybeans to high temperatures.

Exotic genotypes have rarely been used for improvement. PI research has focused on the search for genes related to tolerance to biotic and abiotic stress, while few studies have been conducted to improve yield characters. Therefore, the objective of this study was to select the markers related to soy yield under high temperatures, via associative mappings, to identify sources of tolerance for high temperatures in soybeans.

MATERIAL AND METHODS

The germplasm evaluated was formed by the PI of soybeans from 32 countries, in addition to 15 controls formed by commercial crops and improved lineages from the soybean improvement program of the Genetic Diversity Laboratory, Genetics Department, Escola Superior de Agricultura Luiz de Queiroz, in Piracicaba (São Paulo, SP).

The genotypes were evaluated and planted under field conditions in an experiment in the city of Teresina (Piauí - PI) (05°05’05″ S, 42°47’05″ W, 72 m), classified by the Instituto Nacional de Meteorologia (the National Agrometeorology Institute) as the city with the third highest temperature in Brazil. During the experiment, the mean temperature was 28.2 °C, with a minimum of 22 ºC and a maximum of 34.3 ºC, according to data from a meteorological station near the experimental field.

To evaluate the genotypes, the experiment was conducted in an Alpha Lattice Design of 5 × 19, with three repetitions. The sets were formed by 4 lines of 5 m each and spaced by 0.5 m. Only the two central lines were harvested, as this was considered to be the functional area of each set. The cultivars used were common in the region, following the technical recommendations for the culture.

To phenotype the genotypes, the following characters were evaluated: number of days until flourishment (NDF), measured by the number of days between the day of sowing and the day when 90% of the set reached flowering (R2); start of graining (R5), measured by the number of days from sowing until the start of grain filling in 50% of the set; end of graining (R7), measured by the number of days from the sowing to complete graining of 90% of the set; graining period (GP), measured by the number of days from stage R5 to stage R7; height of the plant in its maturity (HPM), calculated using the mean height of five plants that were in the center of the functional area of the set, measured from the soil to the end of their stem, in centimeters (cm); height of the insertion of the first pod (HIP), calculated using the mean height of the first pod in five of the central plants from the functional area of the set, measured from the soil to the top of the plant; agronomic value (AV), evaluated with a score where 1 indicated plants with the lowest value and 5 indicated those with the highest; lodging, scored in a scale where 5 means a completely lodged plant and 1 means a completely straight plant; grain yield (GY), which is the total mass of seeds produced in the useful set, in grams (g); and 100-seed weight (HSW), which is the weight of 100 seeds in grams (g).

Genomic DNA was extracted from young leaves collected from random plants according to Doyle and Doyle's (1987) extraction protocol. Genotyping was performed using the Affymetrix Platform (180 K Axiom® Soybean Genotyping Array), with 180,961 filtered SNPs. The following filter criteria were applied to the original number of SPNs: DQC of 0.82 or more (dish quality control, a measure of resolution distribution of contrast values), QC call rate (quality control call rate) above 92, average call rate for passing of 97 or higher, and minor allele cutoff of 2 or more. These values were based on recommendations from the Axiom Analysis Suite software.

After filtering 20 thousand SNPs for the 95 samples, 50.08% of the SNPs (10,017) remained. These were classified as PolyHighResolution, that is, good cluster resolution with at least two minor alleles. These 10,017 SNPs were used to analyze the structure of the populations.

To evaluate the characters at phenotyping, an intrablock variance analysis of the lattice with lost sets was carried out using SAS 9.0 software (SAS, 2002). Each character was analyzed separately, considering the effects of treatments as fixed, according to the following statistical model:

$$Yijk = m + gi + rj + bk(j) + eijk$$

Where:
$Yijk$: observed value in the set that received the treatment in block k in repetition j;
m: general mean;
gi: treatment effect i (i=1,2,...,95);
rj: effect of repetition, j (j= 1 and 3);
$bk(j)$: effect of block k in repetition j;
$eijk$: experimental error associated with the observation $Yijk$, assuming that the errors are independent and have a normal distribution.

The genetic structure of the mapping population (matrix Q) was determined using the Bayesian method as proposed by Pritchard et al. (2000), using the software Structure. To calculate the number of subpopulations (k), 10 independent runs were used with k varying from 1 to 10 in a no-admixture model, 10 thousand burn-in, and 100 thousand repetitions in each Markov chain (MCMC), and the highest peak from the Evanno (EVANNO; REGNAULT; GOUDET, 2005) values was used to select the most likely number for k.

The linkage disequilibrium (LD) between pairs of markers was calculated using the determination coefficient r^2, using the permutation test in the TASSEL software (BRADBURY et al., 2007). When the p-value was < 0.01, the loci pairs were considered to have a significant LD.

The tests of the association between the markers and phenotype were carried out using the TASSEL software in a generalized linear model (GML) (BRADBURY et al., 2007), including four phenotypic characters related to yield (AV, HPM, HSW, and GY), the markers, and the Q matrix, according to the statistical model below:

$$Yi = S\alpha + Q\gamma + ei$$

Where:
Yi: value of genotype i;
$S\alpha$: data from genotyping using SNP markers
$Q\gamma$: population structure matrix;
ei: associated experimental error.

The p-value ($p < 0.001$) was used to determine the linkage degree between the locus and the marker and to quantify the magnitude of the influence of the region on the phenotype observed.

RESULTS AND DISCUSSION

The adequate temperature for soybean sowing ranges from 15 to 22 °C during its mature stage (LIU; JIAN; GUANGHUA, 2008). During the experiment, the temperatures were higher than that recommended for soybeans, varying from 29 ºC to 34.4 ºC, characterizing thermal stress during all stages of the culture.

Owing to the loss of some sets, an intrablock lattice analysis was carried out in the lost sets (Table 1) for each character analyzed. All characters evaluated showed a significant difference in the F test ($p \leq 0.05$), indicating the variation between the genotypes studied.

Regarding NDF, the mean was approximately 34 days, indicating that some genotypes presented early flowering, which may have occurred due to the high temperatures and the fact that the experiment was carried out at low latitudes, leading to photoperiodic influences such as early flowering (DIDONET; VITÓRIA, 2006).

Table 1. Intrablock analysis of the variables number of days until flowering (NDF), height of insertion of the first pod (HIP), agronomic value (AV), start of graining (R5), end of graining (R7), graining period (GP), height of the plant in maturity (HPM), lodging (LO), 100-seed weight (HSW), and grains yielded per set (GYS), in an alpha lattice design to select soybean genotypes related to high-temperature tolerance, in an experiment conducted in Teresina, PI, Brazil.

FV	GL	Mean squares									
		NDF	HIP	AV	R5	R7	GP	HPM	LO	HSW	GYS
Gen	94	37.32*	0.05*	88.68*	131.11*	132.14*	0.02*	616.48 *	1.25*	19.31*	0.32*
Bl	54	18.50	0.03	59.12	91.43	96.01	0.02	659.59	1.17	15.84	0.15
Rep	2	14.78	0.04	44.34	62.54	339.94	0.11	7576.80	10.75	11.34	0.37
Er	132	7.52	0.01	17.74	18.45	37.96	0.01	79.10	0.57	9.12	0.16
CV		8.03	10.40	35.33	7.80	7.38	7.69	16.06	33.54	25.23	13.55
Mean		34.14	13.88	2.38	55.10	83.45	28.34	55.37	2.25	11.97	708.57

Early lineages usually start with lower plants and lower first pod insertion. According to Rocha et al. (2012), when sowing occurs in regions with shorter days, the plant is shorter. Board and Harvilhe (1998) state that thermal stress can lead to weaker performance. When coupled with high temperatures, the sowing of soybeans at lower latitudes also leads to shorter plants at maturity, which can be noted from their mean height of 55.37 cm.

Lodging is a common characteristic of soybean PIs. This can be a sign of bad adaptation to the site of the culture (ROCHA et al., 2012), and this characteristic may have been expressed in this experiment by some PIs due to the low adaptability of the sowing at low latitudes.

The diminution in the size of the seed and, therefore, of its weight, can be a consequence of the reduced viability of pollen, diminished production of pollen, difficulties in the growth of the pollen tube, and asynchrony in the stamen and gynoecium (PRASSAD et al., 2003).

According to Thuzar et al. (2010), an increase of a few degrees is enough to significantly diminish soybean yield, due to the diminution of the leaf area and, consequently, of the photosynthetic rate. The mean GY of the genotypes was considered to be quite below the regular soybean values, including those for PIs.

The linkage disequilibrium standard calculated was based on 6,297,225 combinations of 7,434 SNP loci. Based on r^2, 34.06%SNPs exhibited significant linkage disequilibrium ($r^2 < 0.01$). The r^2 values were related to the genetic distance between the markers and the base pairs (Figure 1), which showed that the decay of LD increased as the genetic distance increased. The slow LD decay, detected using the genetic distance mapping, is usually expected in studies of autogamous plants, where the LD extends to hundreds of pairs of bases.

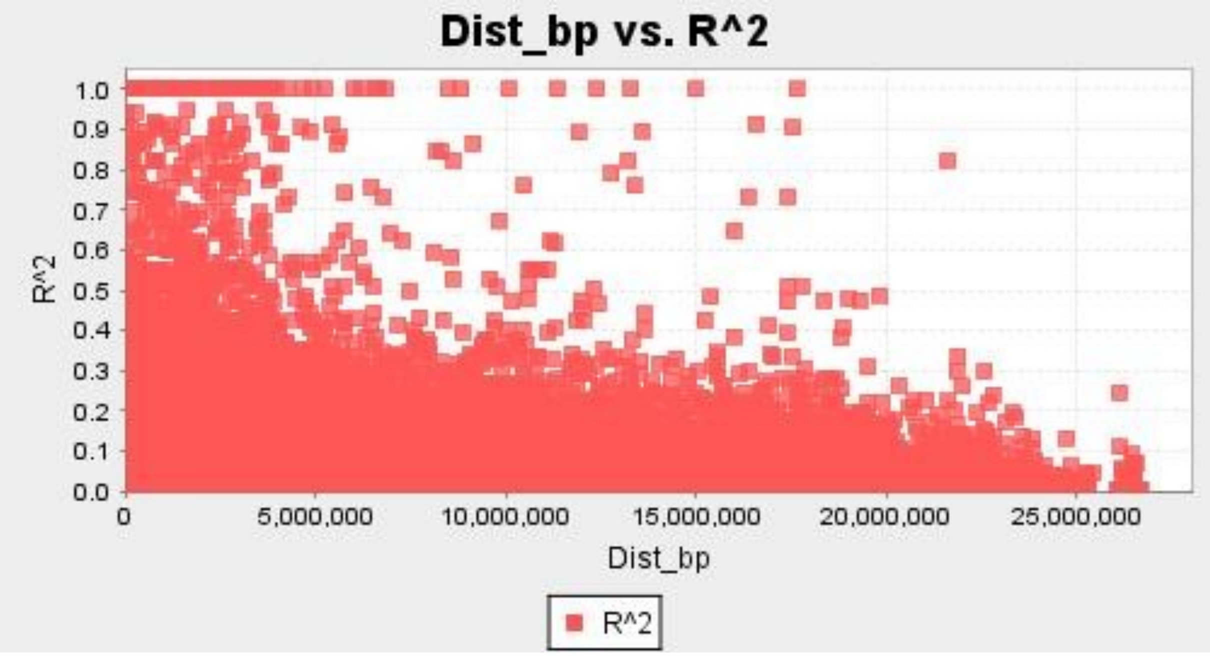

Figure 1. Decay of linkage disequilibrium according to the genetic distance between the marker pairs used in the associative mapping.

The analysis of linkage disequilibrium is particularly important for associative mapping, since this approach mainly detects associations between the marker and the characters of interest indirectly using the LD between both (WU¨RSCHUM et al., 2013). Thus, the selected markers can be used to identify genotypes that have the characters of interest.

Starting with associative mapping using a generalized linear model, 16 significant associations were identified (Table 2) for the four characters related to heat resistance in Teresina (PI). Among the 16 significant associations, four were related to the HPM, AV, HSW, and GY.

Table 2. Markers significantly associated with four characters related to the tolerance to heat.

Character	Marker	Cr.	Position	Character	Marker	Cr.	Position
HPM	AX-90509631	6	44119659	HSW	AX-90389614	4	44128585
HPM	AX-90342778	7	11537628	HSW	AX-90354566	6	50427599
HPM	AX-90469593	14	3420844	HSW	AX-90444326	13	35063263
HPM	AX-90327626	19	46979367	HSW	AX-90409840	13	38706066
AV	AX-90402142	7	3354461	GYS	AX-90521700	11	34930663
AV	AX-90342778	7	11537628	GYS	AX-90333592	16	2712250
AV	AX-90521700	11	34930663	GYS	AX-90523249	17	12874430
AV	AX-90525031	16	13424473	GYS	AX-90327053	17	35263624

The associations related to the HPM were distributed in chromosomes 6, 7, 14, and 19. AV was significantly associated with chromosomes 7, 11, and 16. In HSW, on the other hand, the 19 associations were distributed in chromosomes 4, 6, and 13, but only chromosomes 11, 16, and 17 showed significant associations with the markers.

A high threshold (0.001) was used to detect the associations between the markers and phenotypic characters, since the number of markers identified in this study was high. Hu et al. (2014) advocated for a high threshold when there is a high density of markers. No significant association was found when Bonferroni correction was used.

The great importance of associative studies in plants is the search for genes associated with agronomically important characters that have a quantitative heritage. The main advantages of associative mapping are the type of populations in which it can be used, its high precision, and the possibility of generating inferences for other populations. However, no QTL related to resistance to high temperatures has been found in the soybean database (SoyBase). Furthermore, with the expansion of soybean cultivation in the MATOPIBA region (states of Maranhão, Tocantins, Piauí, and Bahia), the development of new heat stress-tolerant genotypes may be accelerated by including the markers selected in this study in breeding programs in this region.

The 16 associations were found in 14 markers, as AX-90342778 was simultaneously associated with the HPM and to the AV, and AX-90521700 was associated with AV and GY, which suggests pleiotropy, which in this case favors its use by the breeder.

The use of the assisted selection of these 14 markers can be interesting for breeders that aim to augment their improvement programs with materials that respond to high temperatures, aiming to provide an assisted selection for soybeans subjected to high temperatures at low latitudes.

Owing to the lack of associative studies for high-temperature resistance in soybeans, this approach is useful and can be implemented using full -genome associative mapping, which is a way to explore the entire genome in the search for significant associations, with no other requirement than a high density of genomes (RESENDE, 2008), such as the one used in this study. Furthermore, few studies using this approach via candidate genes have shown good results (ZHU et al., 2008).

Although the panel used was small (95 genotypes) and the phenotyping was carried out in a single environment, the high density of markers, especially in euchromatic regions, guarantees that the data are reliable (HWANG et al., 2014; SAÏDOU et al., 2014; ZIEMS et al., 2014). However, further studies are necessary to corroborate the results found with phenotyping in low-income regions' latitudes and under thermal stress caused by high temperatures during the crop cycle.

CONCLUSIONS

The 16 significant associations between the markers and phenotypes identified via associative mapping using the generalized linear model can provide support to breeders that aim to raise the tolerance to high temperatures in improvement programs for soybeans through selection assisted by markers.

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