

ISSN 1678-3921

Journal homepage: [www.embrapa.br/pab](http://www.embrapa.br/pab)

For manuscript submission and journal contents,  
access: [www.scielo.br/pab](http://www.scielo.br/pab)

# Identification of QTLs in the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*) rice population


**Abstract** – The objective of this work was to identify quantitative trait loci (QTLs) associated with the traits grain yield, plant height, and flowering, as well as superior inbred lines resulting from the intersubspecific cross between 'Araguaia' (*Oryza sativa* subsp. *japonica*) and 'Maninjav' (*Oryza sativa* subsp. *indica*) rice population. A population consisting of 234 recombinant inbred lines (RILs) was assessed in two environments and genotyped using single nucleotide polymorphisms (SNPs) and SilicoDArT markers. Twenty-two QTLs accounting for phenotypic variation ranging from 3.94% to 35.36%, were identified as significant, as follows: six for grain yield, five for flowering, and eleven for plant height. New QTLs were consistently identified for height and flowering traits with the SNP marker 12\_22887040, in both environments, and highlighted for assisted selection of early rice varieties. In both environments, the RIL 1572 with the greatest productivity (6,581 kg ha<sup>-1</sup>), precocity of 70 days to flowering, and the lowest plant height (90 cm) is highly recommended for integration into crosses with elite materials from the rice breeding program.




**Index terms:** linkage disequilibrium, molecular marker, recombinant inbred lines.



## Identificação de QTLs na população de arroz 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*O. sativa* subsp. *indica*)

**Resumo** – O objetivo deste trabalho foi identificar QTLs (*quantitative trait loci*) associados às características rendimento de grãos, altura de planta e florescimento, assim como linhagens superiores, na população oriunda do cruzamento inter-subespecífico 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*). Uma população composta por 234 linhagens puras recombinantes (RILs) foi avaliada em dois ambientes e genotipada por marcadores de polimorfismo de nucleotídeo único (SNPs – *single nucleotide polymorphisms*) e SilicoDArTs. Identificaram-se 22 QTLs com variação fenotípica explicada de 3,94% a 35,36%, que foram significativos, conforme a seguir: seis quanto à produtividade de grãos, cinco quanto ao florescimento e onze quanto à altura de planta. Foram encontrados QTLs inéditos quanto à altura de planta e ao florescimento. O marcador SNP 12\_22887040 identificado nos dois ambientes é indicado para a seleção assistida de variedades de arroz mais precoces. Nos dois ambientes, a RIL 1572 – com maior produtividade (6,581 kg ha<sup>-1</sup>), precocidade de 70 dias até o florescimento e menor altura de planta (90 cm) – é altamente recomendada para a integração em cruzamentos com materiais-elite do programa de melhoramento de arroz.

**Termos para indexação:** desequilíbrio de ligação, marcador molecular, linhagens puras recombinantes.

Jéssica Fernanda Ferreira dos Santos Prado<sup>(1)</sup> 

Antônio Carlos Centeno Cordeiro<sup>(3)</sup>   
Alexandre Siqueira Guedes Coelho<sup>(1)</sup>   
Paula Ariele Mendes Ribeiro  
Valdisser<sup>(2)</sup> 

Rosana Pereira Vianello<sup>(2)</sup>  and  
Claudio Brondani<sup>(2)</sup> 

<sup>(1)</sup> Universidade Federal de Goiás, Escola de Agronomia, Rodovia GO-462, Km 0, CEP 74690-900 Goiânia, GO, Brazil. E-mail: [jessica.f.f.santos@hotmail.com](mailto:jessica.f.f.santos@hotmail.com), [alexandre\\_coelho@ufg.br](mailto:alexandre_coelho@ufg.br)

<sup>(2)</sup> Embrapa Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, Caixa Postal 179, CEP 75375-000 Santo Antônio de Goiás, GO, Brazil. E-mail: [paula.valdisser@embrapa.br](mailto:paula.valdisser@embrapa.br), [rosana.vianello@embrapa.br](mailto:rosana.vianello@embrapa.br), [claudio.brondani@embrapa.br](mailto:claudio.brondani@embrapa.br)

<sup>(3)</sup> Embrapa Roraima, Rodovia BR-174, Km 8, CEP 69301-970 Boa Vista, RR, Brazil. E-mail: [antonio.cordeiro@embrapa.br](mailto:antonio.cordeiro@embrapa.br)

✉ Corresponding author

Received  
April 03, 2024

Accepted  
August 13, 2024

**How to cite**  
PRADO, J.F.F. dos S.; CORDEIRO, A.C.C.; COELHO, A.S.G.; VALDISSER, P.A.M.R.; VIANELLO, R.P.; BRONDANI, C. Identification of QTLs in the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*) rice population. *Pesquisa Agropecuária Brasileira*, v.59, e03723, 2024. DOI: <https://doi.org/10.1590/S1678-3921.pab2024.v59.03723>.

## Introduction

Brazilian Agricultural Research Corporation (Embrapa) maintains the largest active collection of rice (*Oryza sativa* L.) germplasm in Brazil, comprising more than 20 thousand rice accessions. From this collection, the core collection (CNAE) of Embrapa Rice & Beans (Embrapa Arroz e Feijão) was established in 2002, serving as a valuable resource for research and genetic breeding efforts (Abadie et al., 2005). This germplasm encompasses the diversity within cultivated rice and less adapted rice landraces capable of crossbreeding and producing fertile offspring (Ramos et al., 2019). Such genetic diversity plays a crucial role in the enhancement of yield, quality, and resilience of rice against both biotic and abiotic environmental stresses. With the increasing frequency of natural disasters affecting agricultural sectors and food security, the integration of long-term strategies into breeding programs is imperative, to explore germplasm banks for the resilience enhancement, as extensively pointed out (Pathirana & Carimi, 2022; Salgotra & Chauhan Jr., 2023).

The exploration of broad crosses in rice, such as those involving genitors of the *indica* and *japonica* subspecies, enables the acquisition and identification of new gene combinations (Zhang, 2020). The divergence between *indica* and *japonica* occurred before domestication, which began approximately 9,000 years ago, resulting in an independent reservoir of genetic diversity (Fragoso et al., 2017). Several studies involving *indica* x *japonica* crosses have been carried out and have reported success in both the identification of superior inbred lines and the identification of molecular markers due to the greater genetic distance between these parents (Seo et al., 2020; Song et al., 2023a). However, crosses between *indica* x *japonica* parents can result in progenies with segregation distortion due to genetic divergence (Wang et al., 2009).

'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjau' (*Oryza sativa* subsp. *indica*) cross was established through a combinatorial capacity study involving 12 Embrapa Rice & Beans genotypes known for their high grain productivity (Ramos et al., 2019). Among the genotypes evaluated in the study, the greatest genetic distance, as determined by simple sequence repeat (SSR) markers, was observed between the Brazilian upland rice cultivar 'Araguaia' and the Asian irrigated cultivar 'Maninjau' (Ramos et al., 2019). The genotyping of inbred lines derived from

*indica* x *japonica* crosses by SNPs allows to attain a large number of markers distributed throughout the rice genome, despite the potential for segregation distortion (Seo et al., 2020).

The objective of this work was to identify quantitative trait loci (QTLs) associated with the traits grain yield, plant height, and flowering, as well as superior inbred lines resulting from the intersubspecific cross between the rice populations of 'Araguaia' (*Oryza sativa* subsp. *japonica*) and 'Maninjau' (*Oryza sativa* subsp. *indica*).

## Materials and Methods

The segregating population evaluated in this work was composed of recombinant inbred lines (RILs) generated from the cross between 'Maninjau' and 'Araguaia', using the single seed descent method (SSD) until the F2:7 generation.

The experiment was carried out in two locations under an irrigated cultivation system: at the Embrapa Roraima experimental station, in the municipality of Boa Vista, in the state of Roraima (RR) (2°48'N, 60°39'W, at 61 m altitude), during the 2017/2018 crop season; and at Fazenda Palmital, owned by Embrapa Rice & Beans, located in the municipality of Goianira, in the state of Goiás (GO) (16°26'S, 49°23'W, at 728 m altitude), during the 2018/2019 crop season. In both locations, the climate and soil classification were, respectively, Aw (Köppen-Geiger's climate classification) and Gleissolo (Santos et al., 2018).

In Boa Vista, 243 recombinant inbred lines (RILs) and 17 controls (including the parents) were evaluated. In Goianira, 247 RILs and 9 controls (including the parents) were evaluated. The total of 251 RILs was evaluated (239 common, 4 specific to Boa Vista, and 8 specific to Goianira, due to seed availability).

The experimental designs used were the lattices 17 x 18, in Boa Vista, and 16 x 16, in Goianira, both with two replicates. The plots consisted of four rows measuring 4 m length, with 17 cm row spacing and sowing density of 75 seed per meter.

The traits evaluated were grain yield, flowering, and plant height. Grain yield was determined after the complete physiological maturation of grains, by converting the weight of grains harvested from each plot into kilograms per hectare. Flowering was assessed by counting the number of days from sowing until 50% of the flowering panicles was reached. Plant

height was estimated after the plant maturation stage, by randomly measuring five plants in the plot, from the main stem in the soil to the end of the panicle.

The phenotypic data were subjected to individual and joint statistical analyses of the environments by using the R version 3.5.3 program (R Core Team, 2019). Estimates of variance components were obtained using the restricted maximum likelihood (REML) method, applying the empirical best linear unbiased predictors (eBLUPs).

Genomic DNA was extracted from young leaves of the 251 RILs and their parents by using the commercial kit DNeasy 96 Plant Kit (Qiagen, Germantown, MD, USA). After sample preparations, DNA was sent to Diversity Arrays Technology (DArT) Pty Ltd (Bruce, Australia) for genotyping. The DArTseq methodology employed generated dominant SilicoDArTs markers (presence/absence) and SNP markers.

Genotypic data filtering and QTL analyses were conducted using the statistical software R version 3.5.3. Bioinformatics filtering steps, including the removal of markers with missing data, distorted segregation, monomorphism, and heterozygous markers, were applied to obtain high-quality SNP data. Mapping was carried out for the traits grain yield, flowering, and plant height, utilizing data from 234 RILs and 8,911 SNPs and SilicoDArTs markers. The method used was a multiple interval mapping and logarithm of the odds (LOD score)  $\geq 3.0$ . The nomenclature of significant QTLs followed the guidelines described by McCouch et al. (1997).

A linkage disequilibrium (LD) analysis was performed by assessing pair-wise markers using the quadratic coefficient of correlation ( $r^2 \geq 0.8$ ) (Bradbury et al., 2007) in the TASSEL 5 program, version 5.2.51 (Glaubitz et al., 2014). Additionally, LD decay was determined using a nonlinear model (Hill & Weir, 1988) in the R software, version 3.5.3. Specific SNP markers flanking the target QTL were placed into haplotype blocks using the Haploview software (Barrett et al., 2005).

## Results and Discussion

The analysis of variance components showed a significant difference between the progenies and controls assessed for most traits, in both the individual and joint analyses of the environments (Table 1). Variance data and estimates of genetic parameters

indicate the existence of sufficient genetic variability, to select superior RILs for the evaluated traits of interest, as described by Idris & Mohamed (2013). Furthermore, the analysis of variance identified a significant interaction between the environment and the RILs, indicating that environmental factors influenced their performance. Similar findings were reported by Ramos et al. (2019), accessing the trait plant height among RILs from the intersubspecific population 'Epagri 108' x 'Irat 122' in Boa Vista and Goianira.

The joint analyses ranking the RILs by adjusted means facilitated the comparison of the performance of the six most productive RILs with the six common controls evaluated across both environments, as well as their parents (Table 2). Regarding grain yield performance, five controls outperformed the six RILs and parents, suggesting that the most productive RILs may not significantly contribute to yield gains in the breeding program similarly to the plant height trait. However, these RILs may offer advantages for precocity, as they exhibited lower flowering values than both controls and parents. Notably, RIL 1572 showed superior precocity, the lowest height, and the second-highest productivity among the other RILs, highlighting its potential for breeding purposes.

The 234 RILs were genotyped for QTL mapping and analyses, with a total of 33,099 markers (15,181 SNPs, and 17,918 SilicoDArTs). The number of polymorphic markers identified in the present work was similar to that found by Phung et al. (2014) for an *indica* x *japonica* cross, in which 25,971 markers (consisting of 10,687 SNPs, and 15,284 SilicoDArTs) were detected across 185 inbred lines. Following necessary filtering procedures, a subset of 8,911 markers (comprising 5,025 SNPs, and 3,886 SilicoDArTs) remained for subsequent analysis. A large reduction (73%) in the number of markers suitable for genetic analysis was observed, which is an important point to consider when carrying out broad crosses in rice. Segregation distortion and a high frequency of missing data are expected, due to the greater genetic incompatibility inherent in such crosses compared to *japonica* x *japonica* and *indica* x *indica* crosses (Guo et al., 2016).

The set of markers spanned a map distance of 1,621 cM, with an average distance of 0.18 cM between markers (Table 3). The mean LD decay, corresponding to a reduction of 50% from the maximum value, predicted by the nonlinear regression ( $r^2=0.46$ ), was

considered high ( $r^2 = \sim 0.22$ ) and reached a physical distance of 4,960.84 Kb. This result suggests the formation of large blocks of haplotypes. However, the identification of these blocks can be the starting point for meta-analyses that can identify conserved regions between different populations and, consequently, identify candidate genes based on their respective aminoacid sequences (Anilkumar et al., 2022). For the *indica* subspecies, the average LD decay typically ranges between 50-200 Kb (Xu et al., 2011), whereas, for the *japonica* subspecies, it is common to observe an average decay greater than 500 Kb (Chen et al., 2013).

Multiple interval mapping analysis identified 22 significant QTLs (minimum LOD score = 3), as follows: 6 for grain yield; 5 for flowering; and 11

for plant height. From the analysis of the genotypic profile of the RILs, in relation to the markers that flanked the peaks of the QTLs, it was found that the majority of alleles with a favorable effect on grain yield came from the 'Maninjau' parent, while those for flowering and plant height came from the 'Araguaia' parent. As observed by Gu et al. (2023), the genetic complementation is prevalent in intersubspecific hybrids. In the present work, this prevalence allowed both parents to contribute favorable alleles for the evaluated characters. The QTLs identified for grain yield on chromosomes 1, 2, 3, 8, and 12 explained from 6.13% to 14.91% of the phenotypic variation, and they contributed from 49.67 kg ha<sup>-1</sup> to 282.33 kg ha<sup>-1</sup> for grain yield of the RILs (Table 4). The complexity of the

**Table 1.** Analysis of variance, estimates of variance components, and genetic parameters for rice (*Oryza sativa*).

Parameter	Location	Effect	Grain yield (kg ha <sup>-1</sup> )	Flowering (days)	Plant height (cm)
p-value	Boa Vista, RR	RIL	2.2e-16***	2.2e-16***	2.2e-16***
		Control	2.2e-16***	2.2e-16***	5.897e-08***
	Goianira, GO	RIL	2.2e-16***	2.2e-16***	2.2e-16***
		Control	2.2e-16***	2.2e-16***	3.978e-14***
	Joint analysis	RIL	1.993e-11***	2.2e-16***	2.2e-16***
		Control	1.0 <sup>ns</sup>	1.0 <sup>ns</sup>	2.2e-16***
		Progeny x environment	2.2e-16***	2.2e-16***	5.57e-10***
		Control x environment	0.00131**	6.433e-10***	0.4613 <sup>ns</sup>
Genotypic variance ( $\sigma^2_g$ )	Boa Vista	RIL	84.1	36.4	57.5
		Control	115.8	147.3	130.6
	Goianira	RIL	115.7	23.1	107.8
		Control	157.7	324.2	344.1
	Joint analysis	RIL	53.0	24.5	69.3
Control	150.2	87.8	277.2		
Residual variance ( $\sigma^2_e$ )	Boa Vista		31.5	5.3	29.9
	Goianira	RIL	66.9	0.9	29.9
	Joint analysis		48.4	3.2	29.8
Phenotypic variance ( $\sigma^2_p$ )	Boa Vista		57.8	20.9	43.7
	Goianira	RIL	91.3	12.0	68.8
	Joint analysis		50.7	13.8	49.5
Heritability ( $h^2$ )	Boa Vista		0.8	0.9	0.7
	Goianira	RIL	0.7	0.9	0.8
	Joint analysis		0.8	0.9	0.9
CV <sub>g</sub>	Boa Vista		16.8	50.6	54.0
	Goianira	RIL	17.9	27.9	91.1
	Joint analysis		10.0	33.8	64.5
CV <sub>e</sub> (%)	Boa Vista		12.1	3.2	5.2
	Goianira	RIL	12.3	1.1	4.6
	Joint analysis		12.4	2.3	4.9
Accuracy	Boa Vista		0.8	0.9	0.8
	Goianira	RIL	0.8	0.9	0.8
	Joint analysis		0.9	0.9	0.9

\*\*\*Significant at 0.1%. \*\*Significant at 1%. <sup>ns</sup>Nonsignificant. CV<sub>e</sub> (%): experimental coefficient of variation; CV<sub>g</sub>, genetic coefficient of variation; RIL, recombinant inbred line from the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjau' (*Oryza sativa* subsp. *indica*) cross.



**Table 2.** Average data adjusted for joint analysis with the six most productive rice RILs (recombinant inbred lines) from the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*) cross, six controls, and genitors.

Material	Grain yield (kg ha <sup>-1</sup> )	Flowering (days)	Plant height (cm)	
Controls	BRS Catiana	10,821	89.5	91.8
	BRS Pampeira	9,694	91.5	93.5
	BRS Tropical	9,194	89.0	93.2
	IRGA 417	8,387	79.5	90.1
	Epagri 108	8,144	93.0	86.3
	BRS Esmeralda	6,152	70.0	96.5
	Average	8,732	85.4	91.9
	SD	6,152	8.9	3.5
RILs	1486	6,581	70.0	108.6
	1572	6,545	69.3	90.0
	1632	6,307	73.5	100.6
	1575	6,197	74.4	116.0
	1686	5,962	71.1	108.1
	1616	5,913	70.0	116.5
	Média	6,251.3	71.4	106.6
	SD	282.6	2.1	10.1
Genitors	Maninjav	7,340	82.8	117.1
	Araguaia	4,034	80.3	107.2

SD, standard deviation.

**Table 3.** Number and distribution of single nucleotide polymorphism (SNP) and SilicoDArT markers obtained from the genotyping of rice RILs (recombinant inbred lines) from the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*) cross, using the DArTseq technology.

Chromosome	SNP	Silico DArT	Total	Total distance (Mbp)	Total distance (cM)
1	907	535	1,442	43	203
2	736	485	1,221	35	175
3	420	245	665	36	194
4	427	307	734	35	133
5	537	307	844	29	132
6	393	286	679	30	137
7	383	399	782	28	151
8	244	203	447	28	117
9	446	342	788	22	92
10	138	230	368	23	93
11	225	339	564	28	94
12	169	208	377	27	100
Total	5,025	3,886	8,911	364	1,621

Mbp, mega base pair. cM, centimorgans.

**Table 4.** Quantitative trait loci (QTLs) identified for rice grain yield (kg ha<sup>-1</sup>) in RILs (recombinant inbred lines) from the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjav' (*Oryza sativa* subsp. *indica*) cross.

Location	QTL	Chr	Position (cM)	LOD	Marker	Block size (Kb)	Gene number	LOD Peak	ADD	PV (%)	FA	AC	Reference
Boa Vista (RR)	GYLD2	2	104.7	3.87	SNP_2_22651753	139	27	LOC_Os02g37540	223.85	6.91	Maninjav	C/G	Marri et al. (2005)
	GYLD8	8	27.8	3.44	SNP_8_3615434	25	5	LOC_Os08g06430	-211.01	6.13	Araguaia	G/A	Miyata et al. (2007)
Goianira (GO)	GYLD1	1	22.2	5.05	Dart_1_4544449	449	64	LOC_Os01g09030	282.33	8.84	Maninjav	DArT	Tian et al. (2006)
	GYLD3	3	9.0	3.72	Dart_3_1309059	412	59	LOC_Os03g03100	-245.18	6.43	Araguaia	DArT	Li et al. (1997)
Joint analysis	GYLD1.1	1	28.0	8.30	Dart_1_5140203	496	63	LOC_Os01g09890	142.92	14.91	Maninjav	DArT	Cui et al. (2002); Hua et al. (2002); Zhuang et al. (2002); Cho et al. (2007)
	GYLD12	12	34.0	4.41	SNP_12_5143767	484	55	LOC_Os12g37290	49.67	7.65	Maninjav	T/C	Xiao et al. (1996)

Chr, chromosome. Position (cM), QTL position on the genetic map. ADD, additive effect. PV (%), percentage of phenotypic variation explained by QTL. FA, favorable allele inherited from the 'Araguaia' or 'Maninjav' parent. AC, SNP allele change.

grain yield trait makes it very difficult to implement an assisted selection based on markers identified by a QTL analysis that involves crosses, locations, and years different from the one in which the data were obtained (Song et al., 2023b). The identified QTLs are located within blocks ranging in size from 25 to 496 Kb, and, despite the identification of SNPs and SilicoDARTs markers within these QTLs, the presence of a large number of genes within the blocks, ranging from 5 to 64 genes, complicates the pinpointing of the specific genes responsible for the observed traits (Tomkowiak et al., 2021). Additionally, the association between the marker allele and the favorable allele of a given gene may become disrupted over successive generations. The larger is the size of the block under consideration, the higher will be the likelihood of recombination occurring within that fragment (Kumar et al., 2020), which can result in the loss of the *cis* relationship between the marker and the favorable allele responsible for the superior phenotype. The QTL intervals related to grain yield were previously identified in the literature, indicating that these genomic regions are hot spots of genes associated with this trait (Table 4). According to Zhang et al. (2024), hotspot regions are important to identify functional genes. This is particularly important for developing molecular markers specifically linked to the target gene (Salgotra & Stewart Jr., 2020), or as a starting point for developing improved rice plants through gene editing (Huang et al., 2021).

The five QTLs for flowering were detected on chromosomes 3 and 6, explaining from 5.73% to 35.36% of the phenotypic variation and resulting in an increase from 0.78 day to 27.48 days in the flowering of the RILs (Table 5). The QTLs on chromosome 3 (FWRG3.1, FWRG3 and FWRG3.2) contributed 27.48%, 34.38%, and 35.36% of the variation, respectively. The favorable effects of QTLs for this trait came from the 'Araguaia' parent, which also exhibited a greater precocity than that of the 'Maninjau' parent. All QTLs were found in blocks spanning from 163 to 412 Kb (51 to 117 genes). In only one QTL, the LOD peak was located within a gene, while in the others, it was in intergenic regions. No common QTL was identified in the two experimental locations nor in the joint analysis, indicating that the markers for assisted selection should be developed for each location. Rice is a short-day plant, originating in tropical areas close to the Equator and, as latitudes

**Table 5.** Quantitative trait loci (QTLs) identified for rice flowering (days) in RILs (recombinant inbred lines) from 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjau' (*Oryza sativa* subsp. *indica*) cross.

Location	QTL	Chr	Position (cM)	LOD	Marker	Block size (Kb)	Gene number	LOD peak	ADD	PV (%)	FA	AC	Reference
Boa Vista, RR	FWRG3	3	8.8	16.33	SNP_3_2382798	347	51	LOC_Os03g04940	- 3.10	27.48	Araguaia	C/A	Dong et al. (2004); Hittalmani et al. (2003)
	FWRG3.1	3	9.0	21.75	Dart_3_1309059	412	59	Intergenic	- 2.64	34.38	Araguaia	DART	Dong et al. (2004); Hittalmani et al. (2003)
	FWRG6	6	26.6	6.55	Dart_6_5080779	163	117	Intergenic	- 1.17	8.85	Araguaia	DART	Yan et al. (2003)
Joint analysis	FWRG3.2	3	8.8	22.43	SNP_3_1226668	412	55	Intergenic	- 27.48	35.36	Araguaia	A/T	-
	FWRG6.1	6	31.3	4.37	Dart_6_5519024	163	117	Intergenic	- 0.78	5.73	Araguaia	DART	Yan et al. (2003)

Chr., chromosome. Position (cM), QTL position on the genetic map. ADD, additive effect. PV (%), percentage of phenotypic variation explained by QTL. FA, favorable allele inherited from the 'Araguaia' or 'Maninjau' parent. AC, SNP allele change.

**Table 6.** Quantitative trait loci (QTLs) identified for rice height (cm) in RILs (recombinant inbred lines) from the 'Araguaia' (*Oryza sativa* subsp. *japonica*) x 'Maninjau' (*Oryza sativa* subsp. *indica*) cross.

Location	QTL	Chr.	Position (cM)	LOD	Marker	Block size (Kb)	Gene number	LOD peak	ADD	PV (%)	FA	AC	Reference
Boa Vista, RR	PTHT2	2	114.6	3.46	SNP_2_24752077	452	57	LOC_Os02g40840	-1.36	4.11	Araguaia	C/G	Tan Zhenbo et al. (1996)
	PTHT3	3	9.0	8.28	Dart_3_1309059	412	59	Intergenic	2.22	10.33	Maninjau	DART	-
	PTHT4.1	4	20.2	4.02	Dart_4_13282199	495	37	Intergenic	1.48	4.80	Maninjau	DART	-
	PTHT4.2	4	131.0	6.01	SNP_4_35015166	499	80	Intergenic	-1.83	7.33	Araguaia	A/G	-
	PTHT9	9	69.1	3.32	SNP_9_18541358	499	74	Intergenic	-1.33	3.94	Araguaia	C/T	-
Goianira, GO	PTHT12	12	73.1	6.10	SNP_12_22887040	474	55	Intergenic	1.85	7.44	Maninjau	C/A	-
	PTHT4.1.1	4	19.8	4.02	Dart_4_7593479	485	21	LOC_Os04g13590	2.32	5.75	Maninjau	DART	-
	PTHT5	5	83.0	3.16	SNP_5_21097152	462	75	Intergenic	2.07	4.49	Maninjau	T/C	-
	PTHT12	12	73.1	10.27	SNP_12_22887040	474	55	Intergenic	3.82	15.64	Maninjau	C/A	-
	PTHT3.1	3	10.2	4.09	SNP_3_1435501	412	59	Intergenic	1.78	5.39	Maninjau	C/T	-
Joint analysis	PTHT4.1.2	4	20.0	3.64	Dart_4_12046739	495	37	Intergenic	1.66	4.78	Maninjau	DART	-
	PTHT4.2.1	4	131.5	3.73	SNP_4_35059308	499	80	Intergenic	-1.66	4.89	Araguaia	T/C	-
	PTHT12	12	73.1	10.56	SNP_12_22887040	474	55	Intergenic	2.90	14.84	Maninjau	C/A	-

Chr., chromosome. Position (cM), QTL position on the genetic map. ADD, additive effect. PV (%), percentage of phenotypic variation explained by QTL. FA, favorable allele inherited from the 'Araguaia' or 'Maninjau' parent. AC, SNP allele change.

increase, the number of days until flowering changes (Zhu et al., 2018). Therefore, the use of site-specific markers for assisted selection for flowering is due to the complexity of the character, regulated by a system of multiple QTLs and with a pronounced environmental effect (Huang et al., 2024).

The 11 QTLs identified for plant height (on chromosomes 2, 3, 4, 5, 9, and 12) explained a phenotypic variation ranging from 3.94% to 15.64%, resulting in an increase of height ranging from 1.33 to 3.82 cm (Table 6). The QTL PTHT12 that explained 14.84% of the variation was detected in both individual and joint analyses. According to the environment, a differential phenotypic behavior can be a major obstacle for QTL mapping (Park et al., 2023). This is a relevant aspect when considering that the PTHT12 QTL may have a chance of success in marker-assisted selection, by detecting a specific genomic region in a multisite experiment. However, the SNP 12\_22887040 that identified this QTL is located within a 474 Kb block containing 55 genes, making it difficult to determine if the same gene influences height control in the evaluated environments. Nevertheless, given the stability of the QTL, the development of a SNP detection system can be carried out for use in assisted selection. If validated, this marker would prove to be a valuable tool for selecting inbred lines during the seedling stage. Except for PTHT2, the other height-related QTLs, along with QTL FWRG3.2 for flowering, are reported in the present study for the first time, representing a new target loci for genomic studies that may improve our understanding of the genetic regulation of these traits in rice.

## Conclusions

1. The recombinant inbred line (RIL) 1572 –resulting from the intersubspecific cross between 'Araguaia' (*Oryza sativa* subsp. *japonica*) and 'Maninjau' (*Oryza sativa* subsp. *indica*) rice population and distinguished by its higher grain yield, precocity, and lowest plant height, in comparison to other RILs – is recommended for inclusion in crosses with elite germplasm of the rice breeding program.

2. Quantitative trait loci were identified for the first time, from which ten for plant height and one for flowering trait.

3. The SNP marker 12\_22887040 that identified the stable QTL PTHT2, explaining 14.84% of the phenotypic variation, is suggested for further validation in the development of a molecular marker for selecting low-height rice varieties.

### Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for financial support and grants to the fifth (process number 310935/2019-9) and sixth (process nº 313688/2021-4) authors; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Finance Code 001), for grant to the first author; and to Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária – Embrapa), for financial support (process nº 23.14.01.008.00.00).

### References

- ABADIE, T.; CORDEIRO, C.M.T.; FONSECA, J.R.; ALVES, R. de B. das N.; BURLE, M.L.; BRONDANI, C.; RANGEL, P.H.N.; CASTRO, E. de M. de; SILVA, H.T. da; FREIRE, M.S.; ZIMMERMANN, F.J.P.; MAGALHÃES, J.R. Construção de uma coleção nuclear de arroz para o Brasil. **Pesquisa Agropecuária Brasileira**, v.40, p.129-136, 2005. DOI: <https://doi.org/10.1590/S0100-204X2005000200005>.
- ANILKUMAR, C.; SAH, R.P.; AZHARUDHEEN, T.P.M.; BEHERA, S.; SINGH, N.; PRAKASH, N.R.; SUNITHA, N.C.; DEVANNA, B.N.; MARNDI, B.C.; PATRA, B.C.; NAIR, S.K. Understanding complex genetic architecture of rice grain weight through QTL-meta analysis and candidate gene identification. **Scientific Reports**, v.12, art.13832, 2022. DOI: <https://doi.org/10.1038/s41598-022-17402-w>.
- BARRETT, J.C.; FRY, B.; MALLER, J.; DALY, M.J. Haploview: analysis and visualization of LD and haplotype maps. **Bioinformatics**, v.21, p.263-265, 2005. DOI: <https://doi.org/10.1093/bioinformatics/bth457>.
- BRADBURY, P.J.; ZHANG, Z.; KROON, D.E.; CASSTEVENS, T.M.; RAMDOSS, Y.; BUCKLER, E.S. TASSEL: software for association mapping of complex traits in diverse samples. **Bioinformatics**, v.23, p.2633-2635, 2007. DOI: <https://doi.org/10.1093/bioinformatics/btm308>.
- CHEN, H.; HE, H.; ZHOU, F.; YU, H.; DENG, X.W. Development of genomics-based genotyping platforms and their applications in rice breeding. **Current Opinion in Plant Biology**, v.16, p.247-254, 2013. DOI: <https://doi.org/10.1016/j.pbi.2013.04.002>.
- CHO, Y.-G.; KANG, H.-J.; LEE, J.-S.; LEE, Y.-T.; LIM, S.-J.; GAUCH, H.; EUN, M.-H.; McCOUCH, S.R. Identification of quantitative trait loci in rice for yield, yield components, and agronomic traits across years and locations. **Crop Science**, v.47, p.2403-2417, 2007. DOI: <https://doi.org/10.2135/cropsci2006.08.0509>.
- CUI, K.-H.; PENG, S.-B.; XING, Y.-Z.; YU, S.-B.; XU, C.-G. Molecular dissection of relationship between seedling characteristics and seed size in rice. **Journal of Integrative Plant Biology**, v.44, p.702-707, 2002.
- DONG, Y.; KAMIUNTEN, H.; OGAWA, T.; TSUZUKI, E.; TERAOKA, H.; LIN, D.; MATSUO, M. Mapping of QTLs for leaf developmental behavior in rice (*Oryza sativa* L.). **Euphytica**, v.138, p.169-175, 2004. DOI: <https://doi.org/10.1023/B:EUPH.0000046799.21410.13>.
- FRAGOSO, C.A.; MORENO, M.; WANG, Z.; HEFFELFINGER, C.; ARBELAEZ, L.J.; AGUIRRE, J.A.; FRANCO, N.; ROMERO, L.E.; LABADIE, K.; ZHAO, H.; DELLAPORTA, S.L.; LORIEUX, M. Genetic architecture of a rice nested association mapping population. **G3 Genes|Genomes|Genetics**, v.7, p.1913-1926, 2017. DOI: <https://doi.org/10.1534/g3.117.041608>.
- GLAUBITZ, J.C.; CASSTEVENS, T.M.; HARRIMAN, J.; ELSHIRE, R.J.; SUN, Q.; BUCKLER, E.S. TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. **PLoS One**, v.9, e90346, 2014. DOI: <https://doi.org/10.1371/journal.pone.0090346>.
- GU, Z.; GONG, J.; ZHU, Z.; LI, Z.; FENG, Q.; WANG, C.; ZHAO, Y.; ZHAN, Q.; ZHOU, C.; WANG, A.; HUANG, T. ZHANG, L.; TIAN, Q.; FAN, D.; LU, Y.; ZHAO, Q.; HUANG, X.; YANG, S.; HAN, B. Structure and function of rice hybrid genomes reveal genetic basis and optimal performance of heterosis. **Nature Genetics**, v.55, p.1745-1756, 2023. DOI: <https://doi.org/10.1038/s41588-023-01495-8>.
- GUO, J.; XU, X.; LI, W.; ZHU, W.; ZHU, H.; LIU, Z.; LUAN, X.; DAI, Z.; LIU, G.; ZHANG, Z.; ZENG, R.; TANG, G.; FU, X.; WANG, S.; ZHANG, G. Overcoming inter-subspecific hybrid sterility in rice by developing *indica*-compatible *japonica* lines. **Scientific Reports**, v.6, art.26878, 2016. DOI: <https://doi.org/10.1038/srep26878>.
- HILL, W.G.; WEIR, B.S. Variances and covariances of squared linkage disequilibria in finite populations. **Theoretical Population Biology**, v.33, p.54-78, 1988. DOI: [https://doi.org/10.1016/0040-5809\(88\)90004-4](https://doi.org/10.1016/0040-5809(88)90004-4).
- HITTALMANI, S.; HUANG, N.; COURTOIS, B.; VENUPRASAD, R.; SHASHIDHAR, H.E.; ZHUANG, J.-Y.; ZHENG, K.-L.; LIU, G.-F.; WANG, G.-C.; SIDHU, J.S.; SRIVANTANEYAKUL, S.; SINGH, V.P.; BAGALI, P.G.; PRASANNA, H.C.; McLAREN, G.; KHUSH, G.S. Identification of QTL for growth-and grain yield-related traits in rice across nine locations of Asia. **Theoretical and Applied Genetics**, v.107, p.679-690, 2003. DOI: <https://doi.org/10.1007/s00122-003-1269-1>.
- HUA, J.P.; XING, Y.Z.; XU, C.G.; SUN, X.L.; YU, S.B.; ZHANG, Q. Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. **Genetics**, v.162, p.1885-1895, 2002. DOI: <https://doi.org/10.1093/genetics/162.4.1885>.
- HUANG, L.; TANG, J.; ZHU, B.; CHEN, G.; CHEN, L.; BU, S.; ZHU, H.; LIU, Z.; LI, Z.; MENG, L.; LIU, G.; WANG, S. QTL epistasis plays a role of homeostasis on heading



- date in rice. **Scientific Reports**, v.14, art.373, 2024. DOI: <https://doi.org/10.1038/s41598-023-50786-x>.
- HUANG, Y.; DONG, H.; SHANG, M.; WANG, K. CRISPR/Cas systems: the link between functional genes and genetic improvement. **The Crop Journal**, v.9, p.678-687, 2021. DOI: <https://doi.org/10.1016/j.cj.2021.03.004>.
- IDRIS, A.E.; MOHAMED, K.A. Estimation of genetic variability and correlation for grain yield components in rice (*Oryza sativa* L.). **Global Journal of Plant Ecophysiology**, v.3, p.1-6, 2013.
- KUMAR, A.; SANDHU, N.; VENKATESHWARLU, C.; PRIYADARSHI, R.; YADAV, S.; MAJUMDER, R.R.; SINGH, V.K. Development of introgression lines in high yielding, semi-dwarf genetic backgrounds to enable improvement of modern rice varieties for tolerance to multiple abiotic stresses free from undesirable linkage drag. **Scientific Reports**, v.10, e13073, 2020. DOI: <https://doi.org/10.1038/s41598-020-70132-9>.
- LI, Z.; PINSON, S.R.M.; PATERSON, A.H.; PARK, W.D.; STANSEL, J.W. Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. **Genetics**, v.145, p.1139-1148, 1997. DOI: <https://doi.org/10.1093/genetics/145.4.1139>.
- MARRI, P.R.; SARLA, N.; REDDY, L.V.; SIDDIQ, E.A. Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. **BMC Genetics Data**, v.6, art.33, 2005. DOI: <https://doi.org/10.1186/1471-2156-6-33>.
- MCCOUCH, S.R.; CHEN, X.; PANAUD, O.; TEMNYKH, S.; XU, Y.; CHO, Y.G.; HUANG, N.; ISHII, T.; BLAIR, M. Microsatellite marker development, mapping and applications in rice genetics and breeding. **Plant Molecular Biology**, v.35, p.89-99, 1997. DOI: <https://doi.org/10.1023/A:1005711431474>.
- MIYATA, M.; YAMAMOTO, T.; KOMORI, T.; NITTA, N. Marker-assisted selection and evaluation of the QTL for stigma exertion under japonica rice genetic background. **Theoretical and Applied Genetics**, v.114, p.539-548, 2007. DOI: <https://doi.org/10.1007/s00122-006-0454-4>.
- PARK, J.-R.; JANG, Y.-H.; KIM, E.-G.; HUR, S.-S.; KIM, K.-M. Quantitative trait loci mapping identified candidate genes involved in plant height regulation in rice. **International Journal of Molecular Sciences**, v.24, art.16895, 2023. DOI: <https://doi.org/10.3390/ijms242316895>.
- PATHIRANA, R.; CARIMI, F. Management and utilization of plant genetic resources for a sustainable agriculture. **Plants**, v.11, art.2038, 2022. DOI: <https://doi.org/10.3390/plants11152038>.
- PHUNG, N.T.P.; MAI, C.D.; MOURNET, P.; FROUIN, J.; DROC, G.; TA, N.K.; JOUANNIC, S.; LÊ, L.T.; DO, V.N.; GANTET, P.; COURTOIS, B. Characterization of a panel of Vietnamese rice varieties using DArT and SNP markers for association mapping purposes. **BMC Plant Biology**, v.14, art.371, 2014. DOI: <https://doi.org/10.1186/s12870-014-0371-7>.
- R CORE TEAM. **R: a language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2019.
- RAMOS, M.R.F.; MENDONÇA, J.A.; VIANELLO, R.P.; MORAIS JÚNIOR, O.P. de; COLOMBARI, J.M.; BORBA, T.C. de O.; CASTRO, A.P. de; BRONDANI, C. Heterosis and combining ability for grain yield and earliness in accessions of a rice core collection. **Functional Plant Breeding Journal**, v.1, art.5, 2019. DOI: <https://doi.org/10.35418/2526-4117/v1n1a5>.
- SALGOTRA, R.K.; CHAUHAN, B.S. Genetic diversity, conservation, and utilization of plant genetic resources. **Genes**, v.14, art.174, 2023. DOI: <https://doi.org/10.3390/genes14010174>.
- SALGOTRA, R.K.; STEWART JR., C.N. Functional markers for precision plant breeding. **International Journal of Molecular Sciences**, v.21, art.4792, 2020. DOI: <https://doi.org/10.3390/ijms21134792>.
- SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.Á. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; ARAÚJO FILHO, J.C. de; OLIVEIRA, J.B. de; CUNHA, T.J.F. Sistema brasileiro de classificação de solos. 5.ed. rev. e ampl. Brasília: Embrapa, 2018. 356p. Available at: <https://goo.gl/ggQJ22>. Accessed on: July 9 2024.
- SEO, J.; LEE, G.; JIN, Z.; KIM, B.; CHIN, J.H.; KOH, H.-J. Development and application of *indica-japonica* SNP assays using the Fluidigm platform for rice genetic analysis and molecular breeding. **Molecular Breeding**, v.40, art.39, 2020. DOI: <https://doi.org/10.1007/s11032-020-01123-x>.
- SONG, J.; CUI, Y.; FAN, H.; TANG, L.; WANG, J. Molecular breeding of Zheyong810, an *indica-japonica* hybrid rice variety with superior quality and high yield. **Agriculture**, v.13, art.1807, 2023a. DOI: <https://doi.org/10.3390/agriculture13091807>.
- SONG, L.; WANG, R.; YANG, X.; ZHANG, A.; LIU, D. Molecular markers and their applications in marker-assisted selection (MAS) in bread wheat (*Triticum aestivum* L.). **Agriculture**, v.13, art.642, 2023b. DOI: <https://doi.org/10.3390/agriculture13030642>.
- TAN ZHENBO, Z.S.L.; SHEN, L.; KUANG, H.; LU, C.; CHEN, Y.; ZHOU, K.; ZHU, L. Identification of QTLs for lengths of the top internodes and other traits in rice and analysis of their genetic effects. **Acta Genetica Sinica**, v.23, p.439-446, 1996.
- TIAN, F.; LI, D.J.; FU, Q.; ZHU, Z.F.; FU, Y.C.; WANG, X.K.; SUN, C.Q. Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. **Theoretical and Applied Genetics**, v.112, p.570-580, 2006. DOI: <https://doi.org/10.1007/s00122-005-0165-2>.
- TOMKOWIAK, A.; BOCIANOWSKI, J.; SPYCHAŁA, J.; GRYNIA, J.; SOBIECH, A.; KOWALCZEWSKI, P.Ł. DArTseq-based high-throughput SilicoDArT and SNP markers applied for association mapping of genes related to maize morphology. **International Journal of Molecular Sciences**, v.22, art.5840, 2021. DOI: <https://doi.org/10.3390/ijms22115840>.
- WANG, S.; TAN, Y.; TAN, X.; ZHANG, Z.; WEN, J.; KOU, S. Segregation distortion detected in six rice F2 populations generated from reciprocal hybrids at three altitudes. **Genetics Research Cambridge**, v.91, p.345-353, 2009. DOI: <https://doi.org/10.1017/S0016672309990176>.
- XIAO, J.; LI, J.; YUAN, L.; TANKSLEY, S.D. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross.

- Theoretical and Applied Genetics**, v.92, p.230-244, 1996. DOI: <https://doi.org/10.1007/BF00223380>.
- XU, X.; LIU, X.; GE, S.; JENSEN, J.D.; HU, F.; LI, X.; DONG, Y.; GUTENKUNST, R.N.; FANG, L.; HUANG, L.; LI, J.; HE, W.; ZHANG, G.; ZHENG, X.; ZHANG, F.; LI, Y.; YU, C.; KRISTIANSEN, K.; ZHANG, X.; WANG, J.; WRIGHT, M.; McCOUCH, S.; NIELSEN, R.; WANG, J.; WANG, W. Resequencing 50 accessions of cultivated and wild rice yield markers for identifying agronomically important genes. **Nature Biotechnology**, v.30, p.105-111, 2011. DOI: <https://doi.org/10.1038/nbt.2050>.
- YAN, C.-J.; LIANG, G.-H.; GU, S.-L.; YI, C.-D.; LU, J.F.; LI, X.; TANG, S.-Z.; GU, M.-H. Molecular marker analysis and genetic basis for sterility of typical *indica/japonica* hybrids. **Acta Genetica Sinica**, v.30, p.267-276, 2003.
- ZHANG, G. Prospects of utilization of inter-subspecific heterosis between *indica* and *japonica* rice. **Journal of Integrative Agriculture**, v.19, p.1-10, 2020. DOI: [https://doi.org/10.1016/S2095-3119\(19\)62843-1](https://doi.org/10.1016/S2095-3119(19)62843-1).
- ZHANG, X.; SUN, J.; ZHANG, Y.; LI, J.; LIU, M.; LI, L.; LI, S.; WANG, T.; SHAW, R.K.; JIANG, F.; FAN, X. Hotspot regions of Quantitative Trait Loci and candidate genes for ear-related traits in maize: a literature review. **Genes**, v.15, art.15, 2024. DOI: <https://doi.org/10.3390/genes15010015>.
- ZHU, H.; LI, Y.; LIANG, J.; LUAN, X.; XU, P.; WANG, S.; ZHANG, G.; LIU, G. Analysis of QTLs on heading date based on single segment substitution lines in rice (*Oryza sativa* L.). **Scientific Reports**, v.8, art.13232, 2018. DOI: <https://doi.org/10.1038/s41598-018-31377-7>.
- ZHUANG, J.-Y.; FAN, Y.-Y.; RAO, Z.-M.; WU, J.-L.; XIA, Y.-W.; ZHENG, K.-L. Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice. **Theoretical and Applied Genetics**, v.105, p.1137-1145, 2002. DOI: <https://doi.org/10.1007/s00122-002-0974-5>.
-