

Development of rice lines with gene introgression from the wild *Oryza glumaepatula* by the AB-QTL methodology

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ABSTRACT - Wild rice of the species *Oryza glumaepatula* is found Brazil and has been used to broaden the genetic basis of irrigated rice populations in Embrapa breeding programs. Objective of this study was to demonstrate and discuss approaches used in the development of *Oryza sativa* lines containing genes transferred from *Oryza glumaepatula*, resulting in introgression lines with a broader genetic basis and high yield. First of all, genes were transferred from the wild species to cultivated rice by the AB-QTL methodology. Eighteen families were selected using QTL analysis and agronomical performance data. After the heterosis test the families CNAi 9020 and CNAi 9024 were selected and submitted to microsatellite marker-assisted selection. Thirty-five lines were then selected with high plant vigor, high tiller and panicle number per plant, high grain yield of the main crop, and a strong regrowth capacity which makes the use of ratoons a feasible alternative.

Key words: irrigated rice, microsatellite markers, lines, grain yield.

INTRODUCTION

The yield levels of irrigated rice in Brazil have been in stagnation since the end of the 80s when the process of substitution of traditional cultivars by modern varieties of short growth type had reached its peak (Castro et al. 1999). One of the main reasons cited for this situation is the narrow genetic base of the planted cultivars, as a result of the intense improvement of modern varieties which reduced the gene pool. According to Rangel et al. (1996), only ten ancestors account for 68% of the gene pool of

the Brazilian varieties of irrigated rice. Considering the most planted cultivars in the main irrigated rice-producing states, the same authors stated that the seven most frequent ancestors in the pedigrees are responsible for 70% of the genes. Similar results were obtained by Breseghello et al. (1999). This high genetic uniformity may trigger serious consequences for the Brazilian rice production.

A limited genetic variability in the populations in selection affects the gain. Diverse studies have reported genetic gains for grain yield smaller than 1% in the

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improvement programs of irrigated rice conducted in Brazil (Soares et al. 1994, Breseghello et al. 1999, Santos et al. 1999, Rangel et al. 2000).

Wild rice species have been used in genetic improvement programs for broader genetic population bases as much as for the transference of specific traits to cultivated varieties. The use of this germplasm is often hampered since crossings with cultivated rice produce hybrids with various levels of sterility. Besides, the progenies present a series of undesirable traits. One of the alternatives of exploring this variability in genetic improvement programs with a comprehensive mission and working with a series of priorities is the adoption of the pre-breeding phase, which means nothing else than the development of populations or lines through crossings and backcrossings of the exotic germplasm with improved lines. The objective is to transfer useful genes to a genetic environment where they can be used more easily by breeders. This whole process becomes more effective when it is monitored by molecular markers and genetic maps, with the main advantages of creating a larger quantity of data and monitoring the traits that are being transferred.

Of the wild rice species that occur in Brazil, *Oryza glumaepatula*, which is autogamous, diploid and has a similar genome AA to that of the cultivated species *Oryza sativa*, is the most auspicious for the use in genetic improvement (Buso et al. 1998, 2001). Objectives of this study are to show and discuss the procedures adopted in the development of *Oryza sativa* lines with gene introgression from the wild species *Oryza glumaepatula* and their use in the yield increase and broadening of the genetic base of irrigated rice populations.

MATERIAL AND METHODS

Gene transference from *O. glumaepatula* to *O. sativa*

Figure 1 shows the scheme used to obtain the lines of rice with genomic fragments of *O. glumaepatula*. Genes were first transferred from the wild to a cultivated species by Brondani et al. (2001, 2002) using the AB-QTL (*Advanced Backcross Quantitative Trait Loci Analysis*) strategy developed by Tanksley and Nelson (1996). The authors realized the transference in the following steps: a) In 1996, a plant of *O. glumaepatula* accession RS-16 from a wild population of the Amazon region (Buso et al. 1998) was crossed with an elite *O. sativa* line, BG 90-2; b) Establishment of four hybrid F₁ plants, confirmed by RAPD (Random Amplified Polymorphic DNA) and SSR (Simple

Sequence Repeat) markers (Cavalheiro et al. 1996), which were backcrossed with BG 90-2 in 1997; c) In 1998, a total of 256 RC₁F₁ plants with favorable phenotypic traits were backcrossed again with BG 90-2 leading to generation RC₂F₁, which was selfed to produce RC₂F₂ seeds. Ninety-three RC₁F₁ families were selected for the construction of the genetic map based on the PCR reaction using 162 molecular markers (155 SSR and seven STS - sequence-tagged-site markers) (Brondani et al. 2001); d) in the crop year 1999/00, 96 RC₂F₂ families, the two genitors (BG 90-2 and RS-16) and the commercial cultivar BR-IRGA 409 (control) were evaluated in the field in an experimental design of randomized complete blocks with three replications. The experiment was conducted on the experimental fields Fazenda Palmital (Goianira, state of Goiás, site 1) and Formoso do Araguaia (Formoso do Araguaia, state of Tocantins, site 2), of Embrapa Arroz e Feijão, using seedling transplanting. The plots consisted of three 3.0 m long rows with 20 plants each. BG 90-2 was planted along the border rows and the RC₂F₂ families in one central row of each plot where data were collected for eleven agronomical traits. The QTL analyses were carried out using phenotypic data and information from the genetic map (Brondani et al. 2002).

Heterosis tests

Eighteen RC₂F₂ families were selected based on the genetic map, the QTL analyses and on the phenotypic data, bulk harvested (RC₂F₃) and advanced to RC₂F₄ in the winter season (May through October 2000) in the state of Tocantins. The 18 RC₂F₄ families plus the controls BG 90-2 and BRS Formoso were evaluated in trials in the crop year 2000/01 in the states Goiás, Roraima and Tocantins to verify whether the increase in the number of panicles and yield were caused by heterosis or by favorable alleles of *O. glumaepatula*. An experimental design of randomized complete blocks with four replications was used; every plot consisted of four 5.0 m long furrows. Seedling transplanting was used in the trials of Goiás and Tocantins and in Roraima the seeds were planted directly into the dry soil.

The mean data of number of panicles and grain yield in kg ha⁻¹ per site and in the mean of the three sites were analyzed statistically and the means compared by the test of Scott and Knott (1974) at 5% probability.

Molecular microsatellite-marker-assisted selection (SSR) in two RC₂F₆ families

Based on the heterosis tests, two highly productive families (CNAi 9920 and CNAi 9924) were selected for their large number of panicles and because various segments

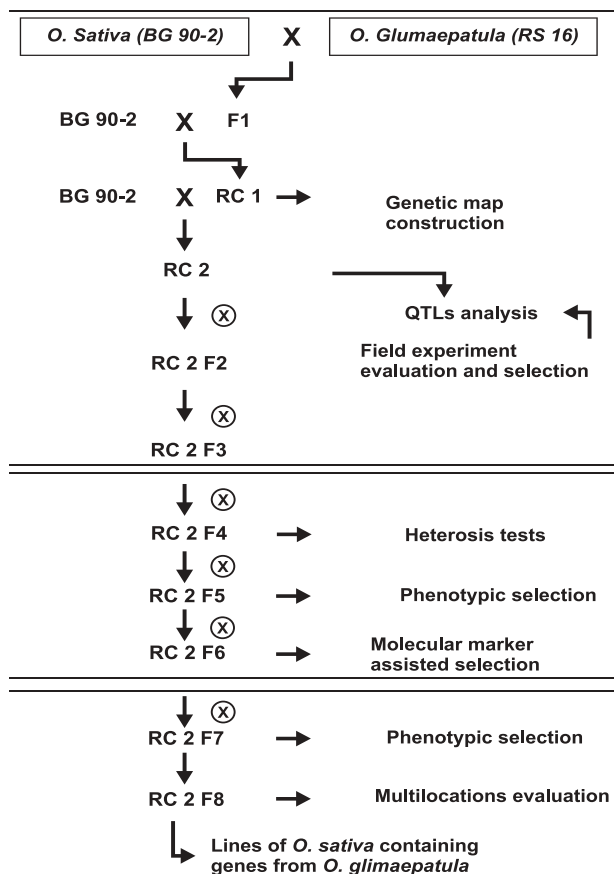


Figure 1. Scheme used in the development of lines of *O. sativa* with gene introgression from the wild species *O. glumaepatula*

in their genotypic constitution contained introgressions of *O. glumaepatula* at estimated percentages of 13% and 8%, respectively. These families were subjected to marker-assisted selection with SSRs.

Two thousand plants of each one of these two families in the RC₂F₅ generation were planted in the field. In the CNAi 9920 and CNAi 9924 families 104 and 168 plants were selected, respectively, considering the number of tillers and panicles and the plant growth type. Ten seeds of each plant in the RC₂F₆ generation were placed in a germinator for 7 to 14 days on germitest paper at 30 °C. The DNA of the germinated seeds was extracted as described by Brondani et al. (1998). Amplification reactions were realized and the amplified loci visualized by electrophoresis in 5% agarose gel stained with ethidium bromide or in 4% acrylamide gel dyed with silver nitrate. To reduce the percentage of the *O. glumaepatula* genome introgressed in the lines CNAi 9920 and CNAi 9924 and to increase the phenotypic value for the traits of interest, SSR markers were selected identified

by the QTL analysis realized by Brondani et al. (2002): OG60, OG99, RM1, RM2, RM4b, RM10, RM11, RM44, RM163, RM223, RM261, OS22 (Table 2).

The remaining RC₂F₆ seeds of the plants selected by molecular markers in the families CNAi 9920 and CNAi 9924 were taken to the field of the experimental station of Formoso do Araguaia, Tocantins, in June, for another evaluation and phenotypic selection. The seeds were sown into a seedbed and the seedlings taken to the field after 30 days where they were planted in plots of eight furrows of 5.0 m of length in a spacing of 0.30 m between furrows and 0.10 m between plants.

Phenotypic evaluation of 35 lines of *Oryza sativa* with gene introgression from the wild species *Oryza glumaepatula*

Thirty-five families, of which eight were transgressive for grain yield and 27 obtained by molecular marker-assisted selection, together with three controls (BG 90-2, Metica 1 and CNA 8502) were evaluated in the experimental design of random blocks with three replications. Every plot was formed by four 5.0 m long furrows spaced 0.30 m and the useful plot area consisted of two central 4.0 m long rows. The sowing density was 100 seeds a meter and the seeds were planted directly into the dry soil. The trial was conducted in Goiás, Tocantins and in Roraima.

The following data were collected in the main crop: mean flowering (Fl), plant height (Ht), lodging (Lg), number of tillers (Ti) and panicles (Pan) per m² and grain yield (Yld) in kg ha⁻¹. In the trial in Goiás data of milling quality were obtained also. Data of the amilose content and gelatinization temperature were collected according to classification criteria of the Centro Internacional de Agricultura Tropical (CIAT 1989) and cooking tests realized. The grains were also classified in long-fine (Lf), long (L), medium (M), and short (C).

In the trials of Goiás and Roraima the lines were evaluated for yield with ratoon cultivation. The plants on the plots were therefore cut after the main crop harvest at a height of about 15 cm and a topdressing of 50 kg of nitrogen ha⁻¹ was applied. The following data were collected: number of tillers panicles⁻¹ in the same sampling area of the main crop, grain yield in kg ha⁻¹, and yield of intact and total grains in the trial conducted in Goiás.

The data of grain yield of the main crop and ratooning underwent the individual and joint analysis of variance and the means were compared by the test of Scott and Knott (1974) at 5% probability.

RESULTS AND DISCUSSION

Heterosis tests

Table 1 shows the number of panicles (Pan) and the grain yield in kg ha⁻¹ of the RC₂F₂ families of the trial conducted in the crop year 1999/00 in Goiás and the number of panicles (Pan) and grain yield in kg ha⁻¹ of the same families in the RC₂F₄ generation of the trial conducted in the crop year 2000/01 in Goiás, Tocantins and Roraima. Significant differences were observed at a probability level of 5% between the means of the families for the traits number of panicles and grain yield in the two generations RC₂F₂ and RC₂F₄. The coefficient of variation for grain yield in the RC₂F₂ generation (21%) was somewhat superior to that obtained for the same trait in the RC₂F₄ generation (Table 1). This can be explained by the fact that one is dealing with a segregant population from a interspecific crossing,

where there is a larger variability among and within families in the RC₂F₂ than in the RC₂F₄ generation.

Of the 18 families selected in the RC₂F₂ generation ten (CNAi 9920, CNAi 9921, CNAi 9922, CNAi 9923, CNAi 9924, CNAi 9925, CNAi 9926, CNAi 9927, CNAi 9928 and CNAi 9929) presented an estimated percentage of the *Oryza glumaepatula* genome varying from 26% (CNAi 9921) to 7.6% (CNAi 9924). All of these produced a larger number of panicles than the genitor BG 90-2. CNAi 9920 presented an increase of 145.8% in the number of panicles per plant in relation to BG 90-2 and possesses 12.6% of the wild species' genome. Eight families (CNAi 9930, CNAi 9931, CNAi 9932, CNAi 9933, CNAi 9934, CNAi 9935, CNAi 9936 and CNAi 9937) did not have detectable chromosome segments of the wild species at the saturation level of the linkage map in use (1 marker for every 10 cM). They were however transgressive for grain yield in relation to control

Table 1. Percentage of *Oryza glumaepatula* in the genome (% *O. glum.*), number of panicles per cova/planting spot (PAN), mean yield in kg ha⁻¹ in Goiás, (GO), Roraima (RR) and Tocantins (TO) and mean of the three sites of the RC₂F₂ and RC₂F₄ families evaluated in the crop years 1999/00 and 2000/01, respectively

Families	<i>O. glum.</i> %	RC ₂ F ₂		RC ₂ F ₄				
		PAN	Yield kg ha ⁻¹	PAN	Yield kg ha ⁻¹			
					GO	Mean	GO	RR
CNAi 9934		8 c	9757 a	13 b	9464 a	9672 a	12148 a	6573 a
CNAi 9937		7 c	9084 a	12 b	9321 a	10256 a	11175 a	6531 a
CNAi 9936		8 c	9660 a	15 a	9255 a	10177 a	10953 a	6635 a
BG 90-2		7 c	8118 a	13 b	9201 a	9828 a	10995 a	6781 a
CNAi 9931		10 b	9967 a	15 a	9178 a	9531 a	11439 a	6563 a
CNAi 9930		9 c	10413 a	13 b	9135 a	9839 a	11161 a	6406 a
CNAi 9935		8 c	9754 a	12 b	9110 a	9643 a	10894 a	6792 a
CNAi 9932		9 c	9940 a	16 a	8929 a	9244 a	10847 a	6698 a
CNAi 9933		9 c	9823 a	12 b	8362 b	8440 b	10314 a	6333 a
BRS Formoso				13 b	8324 b	8350 b	10560 a	6063 b
CNAi 9924	7.6	11 b	8908 a	12 b	8066 b	7897 de	10466 a	5833 b
CNAi 9927	9.8	10 b	6067 b	15 a	7399 c	8050 b	8521 b	5625 b
CNAi 9925	9.0	11 b	7742 a	12 b	6907 d	7923 c	7881 b	4917 c
CNAi 9923	9.8	11 b	6134 b	13 b	6730 d	7370 c	7183 c	5635 b
CNAi 9926	11.9	11 b	6873 b	12 b	6614 d	6925 c	8354 b	4563 c
CNAi 9928	11.3	10 b	5834 b	14 a	6565 d	6236 d	8896 b	4563 c
CNAi 9929	12.5	10 b	7496 a	13 b	6549 d	7798 c	7204 c	4646 c
CNAi 9920	12.6	18 a	8288 a	15 a	5986 e	5849 d	7058 c	5052 c
CNAi 9921	26.0	12 b	3167 c	14 a	5944 e	6181 d	6985 c	4667 c
CNAi 9922	15.8	11 b	2131 c	14 a	5256 f	5409 d	5744 d	4615 c
BR-IRGA 409		7 c	5431 b					
Mean		8	7326	13	7814	8230	9438	5774
CV %		14	21	17	9	11	7	7

Test of means by Scott and Knott (1974) at 5% probability

BG 90-2. CNAi 9930 stood out among the others for producing in the mean 10413 kg ha⁻¹ of grain (Table 1).

The eight transgressive families attained a similar yield in RC₂F₄ to that of the RC₂F₂ generation and the 10 families selected for the number of panicles presented the same performance in both generations (Table 1). This is an evidence that the positive alleles of *Oryza glumaepatula* were responsible for the effects of increase in the number of panicles and yield. Among the ten families with the highest panicle number, CNAi 9924, which also presented high grain yields in the two generations of evaluation, and CNAi 9920, which produced 8288 kg ha⁻¹ in the RC₂F₂ generation were subjected to molecular marker-assisted selection. Based on the QTL mapping analysis in RC₂F₂, plants of these two families that contained the favorable allele for traits related to grain production or alleles in homozygosis for BG 90-2 in regions that contained large fragments of the *O. glumaepatula* genome were selected by assisted selection.

Molecular microsatellite marker-assisted selection (SSR) in two RC₂F₆ families

Favorable alleles for the genitor BG 90-2 and RS-16 were identified (Table 2) in RC₂F₆ plants of the CNAi 9920 and CNAi 9924 families. Of the 104 genotyped plants in the family CNAi 9920, ten presented all favorable alleles in homozygosis for BG 90-2 (plants 27, 55, 60, 62, 74, 78, 82, 88, 89, and 92), and three plants with favorable alleles in homozygosis for RS-16 (plants 1, 25 and 40) (Table 3).

Of the 168 genotyped plants of family CNAi 9924, 118 bearers of both favorable alleles from the genitor BG 90-2 were identified (Table 3). Seventeen plants amongst these were selected for a new evaluation and phenotypic selection in the following generation, totalizing, for both families, 30 RC₂F₆ plants. Three plants selected from family CNAi 9924 (plants 77, 125 and 153) were observed in the RC₂F₇ generation which had very long awn; these were discarded since this trait was inherited from the wild parent *O. glumaepatula*. The evaluation proceeded with these 27 lines plus the eight 8 initially selected lines, amounting to a total of 35 lines.

Phenotypic evaluation of 35 *Oryza sativa* lines with gene introgression of the wild species *Oryza glumaepatula*

The individual and joint analyses of variance for the trait grain yield in the main crop were significantly different at a 5% probability level by the test of Scott and Knott (1974) (Table 4). The coefficients of variation (CVs) of the trials varied from 9% (trial of Goiás) to 23% (trial of Tocantins), considered a good result for experiments conducted in the field and for a quantitative trait such as grain yield.

The mean yield of the three trials was 5885 kg ha⁻¹; the greatest was obtained in the trial of Goiás (8010 kg ha⁻¹) and the smallest in Tocantins (4122 kg ha⁻¹). The lines CNAi 9937, CNAi 9931 and CNAi 9934 were the most productive in the main crop (7398, 7338 and 7135 kg ha⁻¹, respectively) but did not differ significantly from the controls BG 90-2 and Metica 1 (Table 4). Considering only the trials of Goiás and Roraima (Table 5), the mean yield of the two sites in the main crop was 6767 kg ha⁻¹ with the lines CNAi 9937, CNAi 9931 and CNAi 9934 producing around 8000 kg ha⁻¹, and differing significantly from Metica 1, considered the most productive commercial cultivar in the tropical region of Brazil.

The flowering mean of the lines varied from 88 to 95 days, in line with the flowering range of the controls. All lines presented modern plant architecture with a height of about 100 cm, high lodging resistance and upright leaves that stayed green until grain maturation, a trait which is of great importance for being related to high yield in irrigated rice (Table 4).

In the trial conducted in Tocantins, the lines presented high scores for the main rice diseases blast, brown spot, grain spot and leaf scald (Table 4) causing an expressive reduction in the grain yield, evidencing how stressful an environment are the lowland of Tocantins.

The use of the ratoon is a common practice in some countries, as for example the United States (Bollich and Turner 1988), and may be a low-cost alternative to increase the profitability of irrigated rice plantations in Brazil (Santos 1999). Besides the high plant vigor, *O. sativa* lines with gene introgression from the wild species *O. glumaepatula* presented a strong capacity of regrowth, making the use of the ratoons feasible. This trait was evaluated in the trials conducted in Goiás and Roraima, whose data of grain yield of the main crop, of the ratoon, of the sum of both and the number of tillers and panicles are shown in Table 5.

Except for the ratoon in the experiment of Roraima, significant differences were observed by the test of Scott and Knott (1974) at a probability of 5% for the trait grain yield for the main crop, ratoon and the sum of both in the mean of the environments where the trials had been conducted. The lines CNAi 9937, CNAi 9935, CNAi 9931, CNAi 9936, CNAi 9934, and CNAi 9930 produced in the sum of the main and the ratoon crop over 10.000 kg ha⁻¹ (Table 5) and were significantly superior to control Metica 1. Regarding the mean grain yield of both sites and of the main and the ratoon crop separately, these same lines presented statistical superiority to the control Metica 1. Mean grain yields in the ratoon crop of the lines CNAi 9935 and CNAi 9936 were 2627 kg/ha and 2688 kg ha⁻¹ respectively, which made up 34 and 35% of the yield obtained in the main crop.

In general, the evaluated families presented a high number of tillers and panicles m⁻² in relation to the controls

Table 2. Alleles selected by the microsatellite markers used in the individual analysis in the RC₂F₆ generation of the crossing *Oryza sativa* (BG 90-2) x *Oryza glumaepatula* (RS-16)

Line	Marker	Chromosome	Allele to be selected	% Explained phenotypic variation ¹		
CNAi 9920	OG60	4	BG 90-2	17.06 (PLH)		
				23.86 (PFG)		
				20.28 (FGP)		
				31.72 (GYPa)		
	OG99	3	BG 90-2	-		
	OS22	7	BG 90-2	-		
	RM1	1	BG 90-2	48.37 (SPP)		
				39.11 (GYP)		
				42.39 (FGP)		
				31.17 (GYPa)		
RM 2				7	BG 90-2	-
CNAi 9924				RM 4b	11	BG 90-2
	13.25 (HGW)					
	20.82 (GYPa)					
	RM 10	7	BG 90-2	12.95 (HGW)		
	RM 11	7	BG 90-2	15.60 (PLH)		
				17.94 (HGW)		
				13.88 (GYP)		
	RM 44	8	RS-16	32.57 (TNR)		
				30.82 (PNR)		
	RM 163	5	BG 90-2	-		
	RM 223	8	RS-16	32.57 (TNR)		
				30.82 (PNR)		
	OG60	4	BG 90-2	17.06 (PLH)		
23.86 (PFG)						
20.28 (FGP)						
31.72 (GYPa)						
RM 261	4	BG 90-2	12.71 (HGW)			

¹ PLH: panicle length; PFG: percentage of filled grains; FGP: filled grains per panicle; GYPa: yield per panicle; SPP: spikelets per panicle; GYP: yield per plant; HGW: weight of 100 seeds; TNR: number of tillers; PNR: number of panicles

Table 3. Number of homozygote plants that obtained amplification with the markers tested in the lines CNAi 9920 and CNAi 9924

CNAi 9920: 11 tested markers							
Nr of favorable amplified alleles	9	8	7	5	4	3	2
Nr of identified plants	10	14	22	7	13	3	2
CNAi 9924: 2 tested markers							
Nr of favorable amplified alleles	2	1	0				
Nr of identified plants	118	46	4				

BG 90-2 and Metica 1, although there were no significant differences (Table 5). Of the most productive families in the sum of the main and the ratoon crop, CNAi 9935 presented 515 tillers and 511 panicles m⁻². This trait is highly important for irrigated rice, since it contributes to compensate missing

plants in the stand of plantations, requiring a smaller quantity of seeds per hectare and reducing the costs of the plantation.

In general, the lines presented high milling quality and high amilose content and gelatinization temperature. In the cooking tests, most lines presented grains that were slightly sticky after cooking, a trait considered undesirable by Brazilian consumers. Line CNAi 9930 however stood out from the others for presenting a high milling quality in the main (65%) and in the ratoon crop (50%), and loose grains after cooking. This line was also one of the most productive in the sum of the mean yield of the main and the ratoon crop (Table 5). Other lines such as CNAi 9920-1, CNAi 9920-55, CNAi 9920-62, CNAi 9920-78, and CNAi 9924-55 (line with long-fine grain) presented high milling quality in the main and ratoon crop besides presenting loose grains after cooking. This

Table 4. Evaluated data of mean grain yield (GY) in Goiás (GO), Tocantins (TO) and Roraima (RR) and mean yield of the three sites, mean flowering (FLO), plant height (PH), lodging (LODG), panicle blast (PB), brown spot (BS), grain spot (GS), leaf scald (LSC) and phenotypic acceptance (PA) of the *Oryza sativa* lines with gene introgression of the wild species *Oryza glumaepatula*

Treat.	Line	GY	GO	TO	RR	FLO	PH	LODG	PB	BS	GS	LSC	PA
8	CNAi 9937	7398 a	9063 a	6051 a	7080 a	90	99	1	5	5	3	7	5
2	CNAi 9931	7338 a	9292 a	5919 a	6804 a	89	100	1	5	7	5	9	5
5	CNAi 9934	7135 a	9135 a	5439 a	6830 a	90	97	1	7	7	5	9	5
1	CNAi 9930	6998 a	9083 a	4752 a	7159 a	89	99	1	7	5	5	7	5
7	CNAi 9936	6836 a	8365 a	5313 a	6830 a	89	99	1	7	7	5	7	5
36	BG 90-2	6755 a	8781 a	4878 a	6607 a	90	97	1	9	7	7	9	5
37	METICA 1	6696 a	8802 a	5440 a	5845 a	95	106	1	5	5	5	7	3
32	CNAi 9924-117	6610 b	8458 a	5197 a	6174 a	88	94	1	9	7	5	9	5
6	CNAi 9935	6571 b	9031 a	4115 b	6568 a	89	99	1	9	7	3	7	5
21	CNAi 9920-92	6514 b	8469 a	4702 a	6371 a	89	100	1	7	5	5	7	5
4	CNAi 9933	6422 b	8271 a	4546 a	6449 a	89	101	1	7	7	5	9	5
31	CNAi 9924-105	6301 b	8885 a	5053 a	4965 b	88	97	1	5	7	5	7	3
29	CNAi 9924-85	6141 b	8010 a	4239 a	6174 a	87	99	1	7	7	5	9	5
35	CNAi 9924-164	6102 b	8115 a	3596 b	6594 a	88	98	1	7	7	7	7	7
3	CNAi 9932	6065 b	7667 b	4327 a	6200 a	89	96	1	7	7	5	7	5
30	CNAi 9924-92	5999 b	8146 a	4096 b	5753 a	88	98	1	7	7	5	9	3
38	CNA 8502	5975 b	7958 a	4357 a	5609 a	85	98	1	3	3	3	7	5
28	CNAi 9924-61	5928 c	7927 a	4076 b	5780 a	87	95	1	9	9	7	9	5
18	CNAi 9920-82	5806 c	7375 b	4526 a	5518 a	90	93	1	9	7	5	9	5
26	CNAi 9924-41	5774 c	8948 a	2817 b	5556 a	87	93	1	9	7	5	9	3
25	CNAi 9924-37	5771 c	8667 a	3669 b	4978 b	88	98	1	9	7	7	9	5
24	CNAi 9924-23	5688 c	8271 a	3447 b	5346 b	86	95	1	9	7	7	9	3
23	CNAi 9924-15	5598 c	8000 a	3014 b	5780 a	90	97	1	9	9	5	9	5
9	CNAi 9920-1	5491 c	6604 b	4010 b	5858 a	87	95	1	7	7	5	9	7
34	CNAi 9924-142	5430 c	8510 a	4000 b	3780 b	91	97	1	9	7	7	9	3
14	CNAi 9920-60	5387 c	7688 b	3970 b	4503 b	89	97	1	7	7	5	7	5
22	CNAi 9924-3	5374 c	7531 b	4422 a	4170 b	88	94	1	9	7	5	9	5
19	CNAi 9920-88	5345 c	7750 b	3124 b	5162 b	93	99	1	9	7	7	9	5
27	CNAi 9924-55	5344 c	8542 a	3545 b	3945 b	86	91	1	9	7	5	9	5
20	CNAi 9920-89	5246 c	7000 b	4271 a	4467 b	90	96	1	5	7	5	9	5
33	CNAi 9924-138	5219 c	8500 a	3126 b	4032 b	88	95	1	9	7	7	9	3
13	CNAi 9920-55	5044 c	6781 b	3908 b	4442 b	89	94	1	7	7	7	7	5
10	CNAi 9920-25	5040 c	6667 b	3880 b	4574 b	88	88	1	9	9	5	9	7
12	CNAi 9920-40	5022 c	6010 b	3895 b	5162 b	88	96	1	7	7	5	9	7
11	CNAi 9920-27	4960 c	6688 b	2754 b	5438 a	93	99	1	9	7	7	9	5
15	CNAi 9920-62	4940 c	7438 b	2863 b	4519 b	90	97	1	9	7	5	9	5
17	CNAi 9920-78	4708 c	7083 b	2500 b	4542 b	89	96	1	9	7	5	9	5
16	CNAi 9920-74	4677 c	6865 b	2818 b	4348 b	93	95	1	9	7	5	9	5
	Mean	5885	8010	4122	5524								
	CV %	15	9.5	23	16								

Test of means by Scott and Knott (1974) at 5% probability

Table 5. Data of mean grain yield in kg ha⁻¹ of the main (MC) and the ratoon crop (RAT) together and separately and number of tillers (Ti) and panicles (Pan) of the *O. sativa* lines with gene introgression from the wild species *O. glumaepatula* evaluated in trials conducted in Goiás and Roraima

Treat.	Line	Mean of the two sites			Goiás			Roraima			Ti	Pan
		SUM	MC	RAT	SUM	MC	RAT	SUM	MC	RAT		
8	CNAi 9937	10469 a	8071 a	2398 a	12197 a	9063 a	3135 a	8740 a	7080 a	1660 a	404	399
6	CNAi 9935	10426 a	7799 a	2627 a	12875 a	9031 a	3844 a	7978 a	6568 a	1411 a	515	511
2	CNAi 9931	10327 a	8047 a	2280 a	12197 a	9292 a	2906 a	8458 a	6804 a	1654 a	488	485
7	CNAi 9936	10285 a	7597 a	2688 a	12281 a	8365 a	3917 a	8289 a	6830 a	1459 a	463	456
5	CNAi 9934	10282 a	7982 a	2299 a	12052 a	9135 a	2917 a	8512 a	6830 a	1681 a	430	420
1	CNAi 9930	10126 a	8121 a	2005 b	12010 a	9083 a	2927 a	8242 a	7159 a	1084 a	421	419
36	BG 90-2	9981 a	7694 a	2287 a	11854 a	8781 a	3073 a	8108 a	6607 a	1501 a	408	406
32	CNAi 9924-117	9674 a	7316 b	2358 a	11437 a	8458 a	2979 a	7911 a	6174 a	1737 a	468	462
35	CNAi 9924-164	9672 a	7354 b	2318 a	11000 a	8115 a	2885 a	8345 a	6594 a	1751 a	465	463
4	CNAi 9933	9514 a	7360 b	2154 a	11593 a	8271 a	3323 a	7436 a	6449 a	986 a	472	464
31	CNAi 9924-105	9382 a	6925 b	2457 a	12229 a	8885 a	3344 a	6535 b	4965 b	1570 a	543	526
30	CNAi 9924-92	9363 a	6949 b	2413 a	11500 a	8146 a	3354 a	7226 a	5753 a	1473 a	519	515
29	CNAi 9924-85	9347 a	7092 b	2255 a	11020 a	8010 a	3010 a	7675 a	6174 a	1431 a	475	472
21	CNAi 9920-92	9323 a	7419 b	1903 b	10802 a	8469 a	2333 b	7844 a	6371 a	1473 a	490	486
3	CNAi 9932	9220 a	6933 b	2286 a	10968 a	7667 b	3302 a	7471 a	6200 a	1271 a	400	395
38	CNA 8502	9113 b	6783 b	2330 a	11031 a	7958 a	3073 a	7196 a	5609 a	1587 a	448	443
18	CNAi 9920-82	9009 b	6446 c	2563 a	10729 b	7375 b	3354 a	7290 a	5518 a	1772 a	453	437
23	CNAi 9924-15	8902 b	6890 b	2012 b	10635 b	8000 a	2635 b	7170 a	5780 a	1390 a	468	463
28	CNAi 9924-61	8881 b	6853 b	2028 b	10552 b	7927 a	2625 b	7211 a	5780 a	1501 a	449	447
26	CNAi 9924-41	8837 b	7252 b	1585 b	11229 a	8948 a	2281 b	6446 b	5556 a	889 a	477	466
37	METICA 1	8807 b	7323 b	1483 b	9822 b	8802 a	1021 c	7791 a	5845 a	1946 a	425	419
25	CNAi 9924-37	8699 b	6822 b	1877 b	11031 a	8667 a	2365 b	6368 b	4978 b	1389 a	518	515
19	CNAi 9920-88	8637 b	6456 c	2181 a	10500 b	7750 b	2750 a	6774 b	5162 b	1612 a	399	397
20	CNAi 9920-89	8603 b	5733 c	2870 a	11072 a	7000 b	4073 a	6135 b	4467 b	1668 a	411	398
33	CNAi 9924-138	8591 b	6266 c	2325 a	11260 a	8500 a	2760 a	5922 b	4032 b	1890 a	514	499
11	CNAi 9920-27	8530 b	6062 c	2467 a	9843 b	6688 b	3156 a	7217 a	5438 a	1779 a	461	455
15	CNAi 9920-62	8456 b	5978 c	2478 a	10822 a	7438 b	3385 a	6089 b	4519 b	1570 a	451	434
27	CNAi 9924-55	8388 b	6243 c	2144 a	11427 a	8542 a	2885 a	5349 b	3945 b	1404 a	469	457
14	CNAi 9920-60	8385 b	6095 c	2290 a	11072 a	7688 b	3385 a	5698 b	4503 b	1195 a	481	470
34	CNAi 9924-142	8323 b	6145 c	2177 a	11197 a	8510 a	2688 b	5448 b	3780 b	1668 a	569	553
9	CNAi 9920-1	8280 b	6231 c	2049 b	9187 b	6604 b	2583 b	7373 a	5858 a	1515 a	439	430
24	CNAi 9924-23	8279 b	6808 b	1470 b	10239 b	8271 a	1969 b	6319 b	5346 b	973 a	503	493
13	CNAi 9920-55	8264 b	5611 c	2653 a	10572 b	6781 b	3792 a	5956 b	4442 b	1515 a	440	436
16	CNAi 9920-74	8132 b	5606 c	2526 a	10250 b	6865 b	3385 a	6015 b	4348 b	1667 a	444	464
17	CNAi 9920-78	8104 b	5812 c	2292 a	10250 b	7083 b	3167 a	5959 b	4542 b	1417 a	423	410
22	CNAi 9924-3	7807 b	5850 c	1956 b	10416 b	7531 b	2885 a	5198 b	4170 b	1028 a	455	440
12	CNAi 9920-40	7673 b	5586 c	2087 b	8822 b	6010 b	2813 a	6524 b	5162 b	1362 a	443	414
10	CNAi 9920-25	6987 b	5620 c	1366 b	8552 b	6667 b	1885 b	5422 b	4574 b	847 a	522	506
	Mean	8976	6767	2209	10962	8010	2952	6991	5524	1467	463	455
	CV %	12	12	25	9	9	20	15	16	33	18	18

Test of means by Scott and Knott (1974) a 5% of probability

group of lines, originated from the marker-assisted selection, was not the most productive (Tables 4 and 5); in other words, considering only this trait, a phenotypic selection of the 8 initial lines would have been more effective. However, lines with larger fragments of *O. glumaepatula* derived from the families CNAi 9920 and CNAi 9924 presented favorable traits for the grain quality that was not found in the parent line BG 90-2. The effect of the incorporation of these fragments on the quality of the rice grain is being evaluated.

9924-92, CNAi 9924-85, CNAi 9920-92, CNAi 9932, CNAi 9920-1, CNAi 9920-55, CNAi 9920-62, CNAi 9920-78, and CNAi 9924-55 can be used immediately as parents in the improvement program of irrigated rice.

2) Line CNAi 9930 stood out from among the others for uniting a larger number of favorable agronomical traits and could be released as commercial cultivar.

3) The AB-QTL methodology was effective in the development of *O. sativa* lines with gene introgression from the wild species *O. glumaepatula*.

CONCLUSIONS

1) The elite lines of *O. sativa* with gene introgression from the wild species *O. glumaepatula*, CNAi 9937, CNAi 9935, CNAi 9931, CNAi 9936, CNAi 9934, CNAi 9930, CNAi 9924-117, CNAi 9924-164, CNAi 9933, CNAi 9924-105, CNAi

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Desenvolvimento de linhagens de arroz com introgressão de genes de *Oryza glumaepatula*, utilizando a metodologia de AB-QTL

RESUMO - A espécie silvestre *O. glumaepatula*, que ocorre no Brasil, vem sendo utilizada pela Embrapa na ampliação da base genética das populações do melhoramento de arroz irrigado. Assim, o objetivo deste trabalho é mostrar e discutir os procedimentos adotados no desenvolvimento de linhagens de *O. sativa* com introgressão de genes da espécie silvestre *O. glumaepatula*, e a sua utilização no aumento da produtividade e na ampliação da base genética de populações de arroz irrigado. Inicialmente foi realizada a transferência de genes da espécie silvestre para a cultivada usando a metodologia de AB-QTLs. Posteriormente, baseando-se, nas análises de QTLs e nos dados fenotípicos selecionaram-se 18 famílias para aos testes de heterose. Dentre estas as famílias, CNAi 9020 e CNAi 9024 foram selecionadas e submetidas à seleção assistida por marcadores moleculares microssatélites. Ao final do processo foram obtidas 35 linhagens que possuem como principais características agronômicas o elevado vigor de plântula, grande número de perfilhos e panículas por planta, alta produtividade de grãos no cultivo principal e elevada capacidade de rebrota o que torna o aproveitamento da soca uma alternativa viável.

Palavras-chave: arroz irrigado, marcadores microssatélites, linhagens, produtividade de grãos.

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