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CULTIVAR RELEASE

Imidazolinone resistance, yield potential and agronomic performance of the irrigated rice cultivar BRS A706 CL

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Abstract: *Cultivar BRS A706 CL is a new rice cultivar, resistant to herbicides of the chemical group of imidazolinones, with a yield potential of 15,635 kg ha^{-1}. It has intermediate maturation, lodging tolerance, the stay green trait, moderate rice blast resistance, a high milling yield and premium grain quality.*

Keywords: Oryza sativa *L., herbicide resistance, grain yield, breeding, clearfield® production system*

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INTRODUCTION

Brazil produces about 12 million tons of rice (*Oryza sativa* L.) per year on approximately 1.7 million hectares (ANA and CONAB 2020). Irrigated rice is currently cultivated on about 1.3 million ha/year. The improvement in the technological package (i.e., improved variety, enhanced fertilization and weed-control techniques) used for rice production is noticeable. Over the years, the introduction of new production technologies has significantly increased the average grain yield of commercially released cultivars and the water management for irrigation has been streamlined. Over the course of time, these advances have contributed to higher grain yields at relatively unaltered production costs. One of the most relevant technological advances in the sector has been the breeding of improved cultivars. Programs have developed high-yielding cultivars with resistance to the main diseases of the crop and herbicides and grains with excellent industrial and culinary quality.

A key requirement in irrigated rice production is weed control, which directly affects production costs and grain yield. This activity is essential to exploit the maximum yield potential of the cultivars launched by breeding programs. Weeds are mainly controlled chemically, by the application of specific rates of herbicide molecules, by which weed development can be limited without affecting rice production. The most commonly found weed in irrigated rice fields is red rice (*Oryza sativa f. spontanea*), which belongs to the same species as rice. In view

of the highly similar gene repertoire and physiology to those of cultivated rice, the chemical control of this weed is a great challenge.

Chemical weed control by the combination of a herbicide molecule (imidazolinone) with herbicide-resistant rice cultivars was first used in the United States in 2001 (Webster and Masson 2002). The technology was based on gene mutations that made a rice line herbicide-resistant. Other rice lines and plant species without the mutation, including weeds, would die upon contact with the herbicide. Since then, imidazolinone-resistant mutations have been used in breeding programs for the development and commercial release of new cultivars. This is undoubtedly one of the most noteworthy technological milestones in modern rice production.

The first Brazilian imidazolinone-resistant rice cultivar was released in 2002 (Lopes et al. 2004). In Rio Grande do Sul, the main center of rice production of the country, the subtropical irrigated rice area with imidazolinone resistant lines has risen abruptly to 80% of the total acreage within only a few years. Today, imidazolinone is used on nearly the entire production area of irrigated rice in Brazil to control red rice and other weeds. Herbicide resistance and yield are the most important criteria for the acceptance of a new rice cultivar by farmers.

Imidazolinones are molecules with broad-spectrum herbicidal activity, which inhibit the enzyme acetolactate synthase -ALS (or acetohydroxyacid synthase - AHAS). This enzyme processes a key step of the biosynthesis of branched-chain amino acids such as valine, leucine and isoleucine. The enzyme inhibition by the herbicide blocks the metabolic pathway and causes plant death due to amino acid deficiency. Some mutations at specific points of the gene sequence that encodes the enzyme cause changes in its tertiary structure that modify the enzyme/herbicide affinity and thereby cause resistance. Some mutations at the ALS gene therefore have a major technological impact. Imidazolinones are broad-spectrum herbicides, efficient in controlling a variety of weeds, effective at low rates, non-toxic to mammals and highly selective.

The objective of this study was to incorporate resistance to herbicides of the imidazolinone group in cultivars and elite lines of a breeding program of the Brazilian Agricultural Research Corporation (EMBRAPA) and to select the line with the best combination of herbicide resistance, recovery rate of the recurrent parental genome, yield potential and agronomic performance for commercial release. The main experiments to develop cultivar BRS A706 CL are described below.

BREEDING METHOD

Cultivar BRS A706 CL was developed by the backcrossing method, with cv BRS Catiana as recurrent parent and cv Puitá INTA-CL as parental donor of the imidazolinone- resistance mutation. The objective was to develop a new cultivar from BRS Catiana combining the excellent agronomic traits of this cultivar with herbicide resistance. A single cross between cvs BRS Catiana and Puitá INTA-CL was made in 2010 (code CNAx18238), followed by three backcrosses between cv BRS Catiana and plants selected for imidazolinone resistance in each generation. Sequentially, backcrosses were made in 2011, and in the first and second semesters of 2012 (codes CNAx18478, CNAx18767 and CNAx18949, respectively) (Figure 1). All artificial hybridizations were performed in a greenhouse of Embrapa Rice and Beans, in Santo Antônio de Goiás/GO.

Imidazolinone-resistant plants were selected according to the phenotyping protocol originally established for this trait (Rangel et al. 2010). Seeds of segregating generations were germinated in plastic trays in a greenhouse in Goianira/GO. Ten days after seedling emergence, Kifix® herbicide was applied at a rate of 300 g ai ha^{-1} commercial product, plus Dash HC at a rate of 0.5% v/v. Ten days after herbicide application, resistant plants were selected and transplanted into pots for backcrossing. In the 2014/15 growing season, after three backcrossing generations, a progeny test was performed with seeds of the $BC_3F_{2:3}$ generation to detect families with homozygosity for the imidazolinone resistance mutation (Figure 1). The experiment identified four lines (AB161252-RH, AB161253-RH, AB161254-RH and AB161255-RH) as putatively homozygous for the imidazolinone resistance gene. In the 2015/16 growing season, in Preliminary Evaluation Trials conducted at the Experimental Station Fazenda Palmital of Embrapa Rice and Beans, in Goianira/GO, these lines were tested for imidazolinone resistance by Kifix® application, using the nonresistant cv BRS Catiana as control.

Plants of the $BC_3F_{2:3}$ generation of the four selected lines (AB161252-RH, AB161253-RH, AB161254-RH and AB161255-RH) were subjected to molecular analysis to estimate the genome recovery rate (or conversion rate) of cultivar BRS Catiana at the Plant Genetics Laboratory of Embrapa Genetic Resources and Biotechnology. To this end, 600 individual

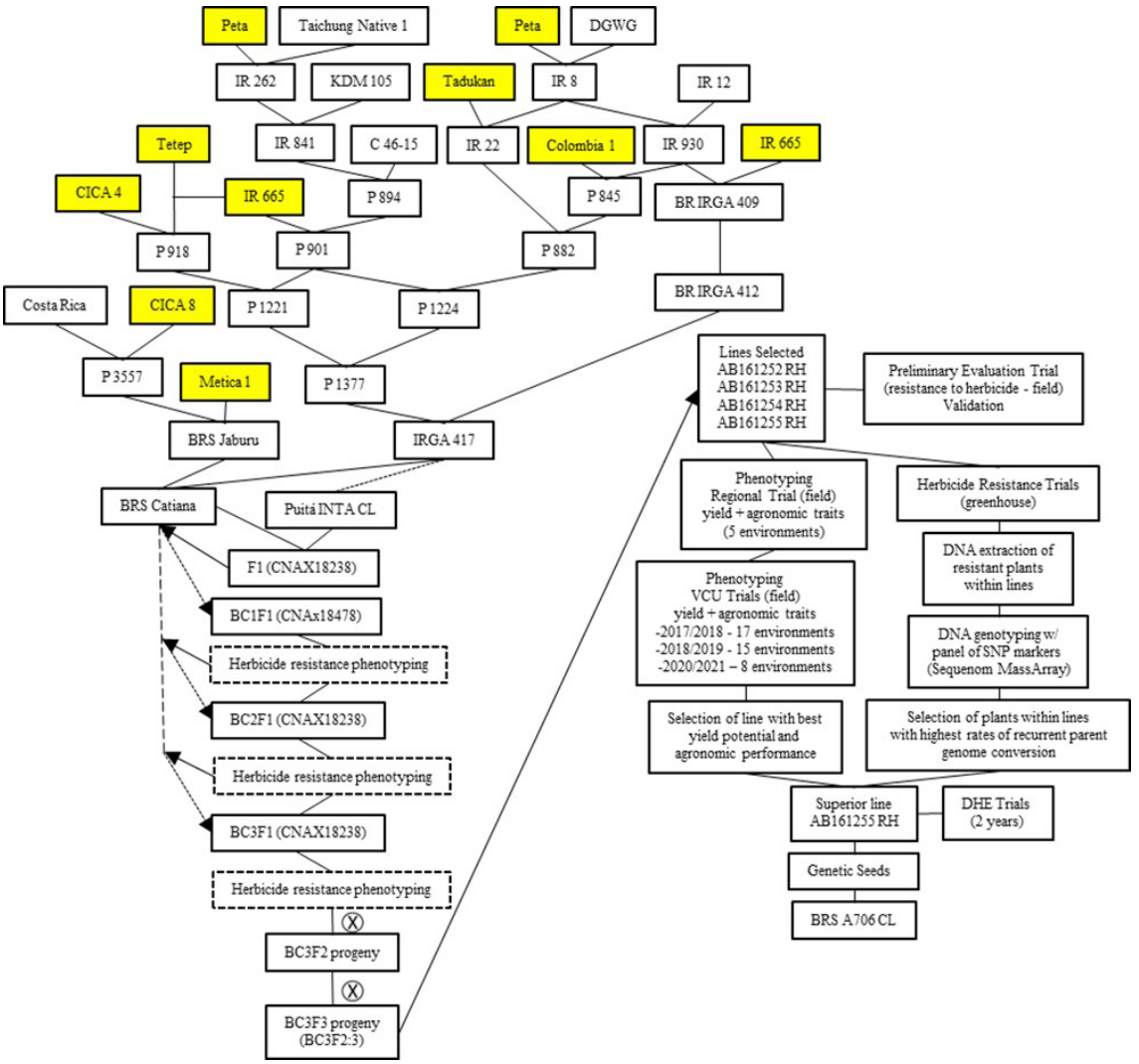

Figure 1. Development of cv BRS A706 CL. Cultivar BRS Catiana was used as recurrent parent in the backcross program. The imidazolinone resistance gene was introgressed from cv Puitá INTA-CL, used as donor parent. Sources of blast resistance are highlighted in yellow (Cica 4, Cica 8, Colombia 1, IR 665, Metica 1, Peta, Tadukan and Tetep).

plants per line were initially subjected to the protocol of imidazolinone treatment in a greenhouse, as described above. One hundred herbicide-resistant plants of each line were selected. These were planted in pots (one seedling per pot) and samples of tender leaves of each plant were collected two weeks after transplanting and subjected to DNA extraction by a modified CTAB method (Ferreira and Grattapaglia 1996). Seeds of each plant were harvested and stored for future use. Extracted DNA was used for genotyping each plant using a panel of 247 SNP markers (Single Nucleotide Polymorphism) via Sequenom MassArray platform. Sequenom MassARRAY® system uses a flexible method of genotyping SNP markers based on the mass of alleles amplified by primer extension via PCR (www.sequenom.com). Allele separation by mass determination was performed by spectrometry (MALDI-TOF). Polymorphisms at marker loci distributed over the 12 rice chromosomes were used to estimate the conversion rate of each chromosome, to identify the plants with the highest recovery rate of the recurrent parent genome. The 10 plants of each line with highest genome recovery rate were selected, some with a recovery rate above 99% and identical in a multilocdus genetic profile. The seeds collected from each of these plants were then sown separately in trays. Ten days after seedling emergence, herbicide Kifix® was applied at a rate of 300 g ai ha^{-1}, plus Dash HC at 0.5 % v/v, to confirm herbicide resistance. Ten days after herbicide application to

the four lines, the plants unaffected by leaf damage, injury or killed by the herbicide effect were planted in the field, in plots with ten 3-m long rows with one plant per hole. Seeds from plants of each line were collected to produce breeder's seed and, later, genetic seed. Genetic seed multiplication for cultivar release was performed after selecting the best of the four lines, based on the results of the molecular analysis and the regional and VCU trials (see below).

In the 2016/17 growing season, seeds of the $BC_3F_{2:3}$ generation of the four herbicide-resistant lines were included in regional trials, at five locations: Goianira/GO (Embrapa Rice and Beans), Flores de Goiás/GO (Emater- GO), Cantá/RR (Embrapa Roraima), Arari/MA (Embrapa Cocais) and Formoso do Araguaia/TO (Unitins Várzea Agro-Environmental Research Center). These tests resulted in the selection of three lines, including line AB161255-RH, which were evaluated in the growing seasons of 2017/18 and 2018/19 in trials to determine the Value for Cultivation and Use (VCU) in Goiás, Maranhão, Piauí, Rio Grande do Sul, Roraima and Tocantins, in a total of 32 environments. The results indicated the superior performance of line AB161255-RH, which was selected for release under the name BRS A706 CL. In the 2020/21 season, cv BRS A706 CL was used as control in the VCU trials in Goiás and Tocantins, in eight environments. These data were also taken into consideration to compute the means of agronomic traits. In compliance with the requirements of Article 22 and the single paragraph of Law of Cultivar Protection # 9,456, of April 25, 1997, line AB161255-RH was also tested in Distinguishability, Homogeneity and Stability (DHE) tests for two years at the Experimental Station Fazenda Palmital of Embrapa Rice and Beans, in Goianira/GO.

PERFORMANCE CHARACTERISTICS

The new cultivar represents a promising option for farmers with regard to the control of weedy rice affecting rice crops, since BRS A706 CL is genetically resistant to herbicides of the chemical group of imidazolinones. These herbicides have pre- and post-emergence action and do not only control dicots, but also grasses and sedges. The herbicide application rates and times must strictly follow the manufacturer's instructions for each commercial product, to avoid the development of resistant weeds due to incorrect use. In a conventional production system, other herbicides than imidazolinones can also be used for cv BRS A706 CL, as part of an optional strategy for the rotation of herbicides with different mechanisms of action, to reduce the selection pressure for the emergence of resistant weeds in the crop. In this regard, the excellent agronomic traits of the cultivar stand out to the detriment of its resistance to imidazolinones.

Cultivar BRS A706 CL was evaluated during three growing seasons in the national VCU trial network of Embrapa in areas with tropical (Goiás, Maranhão, Piauí, Roraima and Tocantins) and subtropical climates (Rio Grande do Sul), where it was compared with control cultivars whose performance is widely recognized in each respective region. The traits grain yield, days to flowering and plant height were evaluated in all trials, while lodging and disease resistance were recorded in environments where the local conditions favor their occurrence. In Rio Grande do Sul, for example, blast (*Pyricularia grisea* (Cooke) Sacc.) was not observed in the VCU trials, but only a slight incidence of brown spot and grain spot, without marked differences between the evaluated lines.

Cultivar BRS A706 CL proved to be widely adaptable for cultivation in different states of Brazil under flood irrigation. The cycle length is intermediate, with about 128 days from seedling emergence to grain maturation (about 96 days between germination and flowering), which may vary according to different management conditions and the cultivation environment, from 112 days in low latitude regions, as in Roraima, to about 135 days in high latitude regions, as in Rio Grande do Sul. The plant height of this cultivar is similar to that of the controls (mean of 99 cm). In terms of disease resistance in the VCU trials, cv BRS A706 CL was similar to cvs BRS Catiana and BRS Pampeira, especially with regard to leaf and neck blast (Table 1).

In the VCU trials, the yield potential of the new cultivar was high (15,635 kg ha^{-1} in the 2020/21 season, in Goiás, Experimental Station Fazenda Palmital of Embrapa Rice and Beans) and mean yield in the subtropical region of 11,022 kg ha^{-1}, which was statistically similar to that of cv BRS Pampa CL (10,315 kg ha^{-1}). The mean yield of cv BRS A706 CL across Brazil was estimated at 8,798 kg ha^{-1} (Figure 2).

Plants of cv BRS A706 CL remain green in the field even when the panicle is already at an advanced stage of maturation. This stay-green trait can offer two basic advantages for production: the first is that carbohydrate translocation is possible for a longer period, since plants are able to carry out photosynthesis and increase yield per hectare even at the end of

Table 1. Agronomic performance of BRS A706 CL and controls: 1) Means of agronomic traits in the trials of Value for Cultivation and Use (VCU) in the growing seasons of 2017/18 (15 locations), 2018/19 (17 locations), and 2020/ 21 (8 locations), in Goiás, Maranhão, Piauí, Rio Grande do Sul, Roraima and Tocantins. 2) Resistance to blast: means of leaf blast recorded in the VNB (Viveiro Nacional de Brusone) trials, carried out in 40 environments, in the 2017/18, 2018/19 and 2020/21 growing seasons. 3) Means of grain quality parameters recorded in VCU trials in the 2020/21 growing season, in Goiás and Tocantins; and in the VNQ (National Grain Quality Nursery) trials, at the Experimental Station Fazenda Palmital, Embrapa Rice and Beans, Goianira/GO, in the 2018/19 and 2020/21 growing seasons

Cultivars	1) VCU – Agronomic traits								2) Resistance to rice blast			3) Grain quality						
	DF	PH	LO	LB	NB	BS_p	ESC	GS_p	[1]M	[1]MSc	[1]F≤3 (%)	L	W	L/W	CHI	WGY	AAC	GT
BRS A706 CL	96	99	1.0	2.1	2.2	2.2	2.1	2.2	2.35	8.00	76	7.24	1.88	3.84	12.9	66.8	23.9(I)	7(B)
BRS Catiana	96	97	1.0	2.2	1.9	2.0	2.2	2.1	2.45	7.00	70	-	-	-	-	-	-	-
BRS Pampeira	96	102	1.2	2.1	2.1	1.9	2.2	2.0	2.21	8.00	72	7.13	1.94	3.68	12.9	67.5	23.0(I)	7(B)
IRGA 424 RI	96	102	1.2	2.1	2.1	1.9	2.2	2.0	3.47	9.00	53	6.67	1.96	3.41	15.5	67.8	24.9(I)	7(B)

Agronomic Traits: DF: days to flowering (days); PH: plant height (cm); LO: lodging (scores from 1 "all plants upright" to 9 "all plants bent over"); and LB, NB, BSp, ESC and GSp: reaction to leaf and neck blast, brown spot, leaf scald and grain spot, respectively (scores from 1 "absence or low disease symptoms" to 9 "very high disease symptoms"). Blast resistance: means (M), maximum scores (MSc) and frequencies of scores ≤3 (F≤3) of leaf blast. [1]Scores from 0 to 9, with 0 for plots with "no symptoms" and 9 for plots with "disease symptoms on more than 50% of the leaf area" (Pinheiro et al. 2009). Grain Quality: L, W and L/W: grain length (L) and width (W) and L/W ratio, respectively; CHI: chalkiness index (%); WGY: percentage of whole grains (%); AAC: apparent amylose content (%, I "intermediate"); GT: gelatinization temperature (scores, B "low").

the cycle. The second advantage is lodging tolerance, as the green stems are stronger and make the plants more resistant to lodging.

Cultivar BRS A706 CL was also evaluated for rice blast resistance, caused by *Magnaporthe oryzae*. For three growing seasons (2017/18, 2018/19 and 2020/21), the cultivar participated in the Viveiro Nacional de Brusone (VNB – National Rice Blast Nurseries) trials, conducted with research partners at different locations across the Brazilian territory, in a total of 40 environments. The cultivar proved moderately resistant to rice blast, with a disease reaction similar to that of cvs BRS Catiana and BRS Pampeira (mean scores < 3, maximum scores 7 - 8); and resistance superior to that of cv IRGA 424 RI (Table 1). However, in general, the new cultivar had better blast resistance reactions than the controls, since scores were equal to or lower than 3 in 76% of the trials, which indicate resistance, while scores above 3 indicate susceptibility (Table 1). These results may be explained by the fact that eight known sources of blast resistance (Cica 4, Cica 8, Colombia 1, IR 665, Metica 1, Peta, Tadukan and Tetep) participated in the pedigree of cv BRS A706 CL, and together represent 70% of its genetic base (Figure 1).

Grain quality was analyzed at the Grain and Byproducts Laboratory of Embrapa Rice and Beans in a device called S 21 Statistical Rice Analyser V. 4.0 (LKL Tecnologia), by which the grain translucency and whole grain percentage can be determined, which revealed that the new cultivar has premium quality (long and thin) grains. This was inferred from the fact that the processed grains have a mean length of 7.24 mm, width of 1.88 mm and length/width ratio of 3.84. The grains are highly translucent and white and have a low

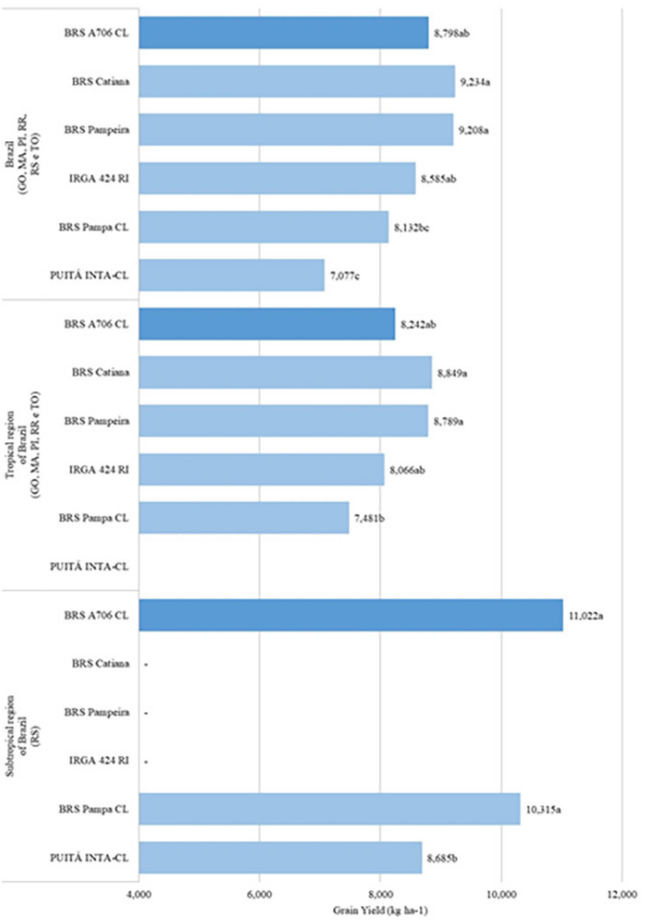

Figure 2. Mean grain yield (kg ha^{-1}) of cv BRS A706 CL and controls, in the trials of Value for Cultivation and Use (VCU) in the growing seasons of 2017/18 (15 locations), 2018/19 (17 locations) and 2020/21 (8 locations), in the States of Goiás, Maranhão, Piauí, Rio Grande do Sul, Roraima and Tocantins. Within each cluster, means followed by the same letter do not differ significantly by the Tukey test, at 5% probability.

total chalkiness area (12.9%), i.e., similar to cv BRS Pampeira and better than cv IRGA 424 RI (Table 1). The levels of the apparent amylose content (23.9%) and gelatinization temperature (grade 7) of cv BRS A706 CL are intermediate and low, respectively. For the culinary properties, the cultivar meets quality expectations of the average Brazilian consumer, with cooked grains that are loose and soft, confirmed in laboratory cooking tests that simulated home cooking for a sensory analysis of the cooked rice texture (Bassinello et al. 2004).

Milling yield is another essential trait to determine the commercial value of rice. According to the mean data of VCU trials, the whole grain yield (WGY) of cv BRS A706 CL was 66.8% (Table 1), i.e., very similar to the control cvs BRS Pampeira (67.5%) and IRGA 424 RI (67.8%). In another experiment, carried out in the 2019/20 season, at the Experimental Station Fazenda Palmital of Embrapa Rice and Beans, using cvs BRS A706 CL, BRS Catiana and BRS Pampeira as controls, five grain samples were collected at 25, 32, 39, 46 and 53 days after flowering to determine grain moisture and whole grain yield. The results showed that the potential of cv BRS A706 CL for maximum WGY (63.2%) was highest when humidity at harvest was around 20%. Even when grain moisture at harvest was between 24% and 16%, the WGY of cv BRS A706 CL was higher or similar to that of cvs BRS Catiana and BRS Pampeira. Therefore, cv BRS A706 CL offers farmers a significant economic advantage due to its stability of whole grain yield, which allows for an extended harvest window, without substantially affecting production profitability.

FOUNDATION SEED PRODUCTION

An excellent yield potential and agronomic performance of line AB161255-RH were observed in the Regional and VCU trials. Seeds were collected from 10 $BC_3F_{2:3}$ plants with the highest conversion rate based on molecular markers. Each family was rigorously screened to eliminate any atypical plants. Seeds were bulk-harvested to obtain foundation seed, which was used for seed multiplication. Cultivar BRS A706 CL is an excellent option for irrigated rice cultivation in the tropical (Tocantins, Roraima, Maranhão and Goiás) and subtropical (Rio Grande do Sul and Santa Catarina) regions of Brazil. It is suitable for both dry soil and pre-germinated no-tillage cultivation systems. The cultivar is resistant to herbicides from the chemical group of imidazolinones, has high grain yield potential, medium maturation cycle, tolerance to lodging, the stay green trait, moderate rice blast resistance, high milling yield, premium grain quality and is recommended for industrial and culinary use. The new cultivar is registered and protected by the Ministry of Agriculture, Livestock and Supply (MAPA) as BRS A706 RH, under no. 47619 in the National Cultivars Registry (RNC), and no. 20210283 by the National Service for Cultivar Protection (SNPC-MAPA). Information and adaptation regions with planting indications of Federal Units can be obtained at the CultivarWeb system of MAPA. Cultivar BRS A706 CL is a new contribution of Embrapa to strengthen rice production and sustainability in Brazil.

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