CULTIVAR RELEASE

BRS A504 CL: a new herbicide resistant upland cultivar with high quality grain

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Abstract: *BRS A504 CL is a new rice cultivar, resistant to herbicides from the imidazolinone chemical group, developed for the Clearfield Production System for upland rice in Brazil. The cultivar has excellent grain quality and yield potential of 8,188 kg ha^{-1}.*

Keywords: Oryza sativa *L., imidazolinone chemical group, grain yield, genetic improvement, clearfield production system*

INTRODUCTION

Brazil is the main consumer and producer of rice outside the Asian continent. Rice (*Oryza sativa* L.) is produced in Brazil in two cultivation systems: irrigated and upland (also known as rainfed rice). These cultivation systems vary in planting methods and productive arrangements according to the environment. The irrigated system is concentrated is some regions (Rio Grande do Sul, Santa Catarina and Tocantins), while the upland system is largely dependent on rainfall irrigation and scattered in many areas of the country. However, there is great potential for planting upland rice in center pivot irrigation systems in rotation with other crops. The main producing states of upland rice are Mato Grosso, Maranhão, Pará, Rondônia and Piauí (Wander et al. 2021). There are around 180 thousand rice-producing properties in Brazil and the vast majority has an area of less than ten hectares (Santana et al. 2022).

For many years, especially during the expansion phase of the agricultural frontier in the Cerrado region, the area planted in the upland system reached significant numbers, culminating in more than 4.6 million hectares in 1986. The extensive planted area resulted in part from the opening of new areas for agricultural exploration. Since the 1990´s, however, there has been a drastic reduction in the importance of this rice cultivation system in Brazil. Until the end of the 1980s, the area planted with upland rice represented 80% of the total rice cultivated area in Brazil. In 2022, however, the upland rice cultivated area was less than 7% of the total, representing only 310 thousand hectares (IBGE 2023).

Despite the efforts of agricultural research that contributed to practically doubling the average yield of upland rice from 1,107 to 2,347 kg ha^{-1} and releasing

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cultivars with yield potentials above 8,000 kg ha^{-1}, the average increase in yield was not enough to compensate for the drastic reduction in the planted area (Wander et al. 2021). Even so, Brazil is probably one of a few countries in the world where upland rice plays an important role in the internal supply of this cereal to the population, acting as a price regulator, favoring a better distribution of rice cropping areas, easing the access of consumer regions to the farmers, mitigating the risks of dependence on just one production system. Upland rice is also an important income alternative for a large number of small farmers, such as in the State of Maranhão, where the vast majority of rice production comes from small properties (Santana et al. 2022).

One of the main obstacles to achieving yield potential is weed control, especially in the initial stages of the crop when weed competition decreases plant development. Crop fields infested with weeds can lead to considerable yield losses, regardless of the production system used. The main weed of irrigated rice crops is spontaneous red rice (*Oryza sativa* f. *spontanea*). In the upland production system, however, the occurrence of red rice is not important. Furthermore, rotating upland rice with soybeans and bean, which use different herbicide molecules, could facilitate weed control in the upland production system. So far there are only two upland rice cultivars (BRS A501 CL and AN 9005 CL) resistant to herbicides available for planting in Brazil. The development of new herbicide-resistant and high yield cultivars with superior grain quality will offer improved options for the farmer and may significantly contribute to the increase in upland rice planting area. The simple insertion of herbicide-resistant cultivars in no-tillage central pivot irrigation systems in rotation with soybeans and bean or in areas of recovery of degraded pastures could represent an excellent alternative for farmers to increase upland rice production and yield, what could positively impact their income (Rangel et al. 2022).

One option for controlling weeds in rice is the use of cultivars resistant to herbicides from the imidazolinone chemical group (IMI). IMI are molecules with wide broad-spectrum herbicidal activity, which inhibit the enzyme acetolactate synthase (ALS) and, consequently, the synthesis of branched-chain amino acids, such as valine, leucine and isoleucine. Deficiency of these amino acids causes plant death. It is a low toxicity herbicide for mammals as this metabolic pathway is typical of plants. It is also effective in low volume, highly selective and considered environment safe as compared to other chemical groups of herbicides.

One of the main criteria for adopting a new rice cultivar in Brazil is herbicide resistance. The objective of this work is to describe the method used to obtain a new rice cultivar resistant to IMI herbicides and describe its agronomic and morphological characteristics. The cultivar BRS A504 CL was developed by Embrapa and is recommended for cultivation in the clearfield production system for upland rice in Brazil.

BREEDING METHOD

The BRS A504 CL cultivar was developed by the backcross method using BRS Esmeralda as a recurrent parent, and Puitá INTA CL as a parent donor of the herbicide resistance gene for the imidazolinone chemical group (IMI). The objective of the backcross program was to develop a new cultivar derived from BRS Esmeralda, adding resistance to IMI herbicides to the excellent agronomic traits of this upland cultivar developed by Embrapa. To this end, in 2010 a simple cross was carried out at Embrapa Rice and Beans, Santo Antonio de Goiás/GO between BRS Esmeralda and Puitá INTA CL, coded as CNAx18247, followed by three backcrosses between plants selected for resistance to IMI herbicides and the recurrent parental (BRS Esmeralda). The backcrosses occurred sequentially in 2011, and in the first and second half of 2012, receiving the respective codes CNAx18486, CNAx18776 and CNAx18958 (Figure 1). All

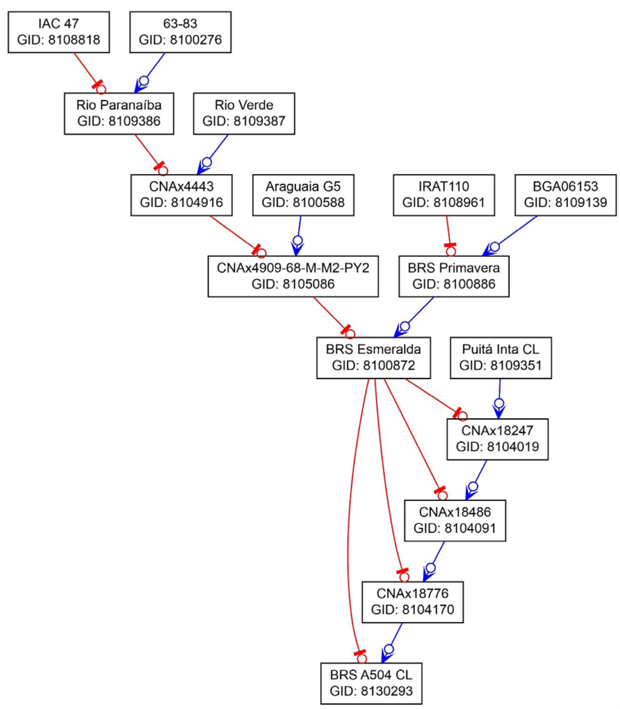

Figure 1. Genealogy of the BRS A504 CL cultivar. GID = Germplasm Identification number.

artificial hybridizations were performed in a greenhouse at Embrapa Arroz e Feijão, in Santo Antônio de Goiás/GO.

The selection of plants resistant to imidazolinones was carried out according to the protocol originally established for phenotyping for this trait (Rangel et al. 2010). The seeds of the segregating generations were germinated in plastic trays in a greenhouse in Goianira/GO and 10 days after the emergence of the seedlings, the herbicide Kifix® was applied with a dose equivalent to 300 g of the commercial product per hectare, plus Dash with a dose of 0.5% v/v. Ten days after applying the herbicide, resistant plants were transplanted into pots to be backcrossed. The backcross progeny tests were carried out in the RC3F2:3 generation (2014/2015 season) and six lines were selected (CNAx18958-1-8, CNAx18958-2-2, CNAx18958-3-2, CNAx18958-4-3, CNAx18958-6-3 and CNAx18958-6-7) putatively homozygous for the resistance allele. These lines were evaluated in the 2015/2016 season for herbicide resistance and agronomic traits in the Regional Trials for the evaluation of upland rice lines in several environments in Brazil. Furthermore, these lines were validated under screen house conditions for resistance to IMI herbicides upon application of Kifix® and using BRS Esmeralda as a control.

Among the six resistant lines obtained from the backcrosses, two were selected based on the results of herbicide resistance trials and evaluations of agronomic characteristics in the field (see below): AB162666-RH (CNAx18958-6-7) and AB162664-RH (CNAx18958-3-2). Remaining seeds from the RC3F2:3 generation of the two selected lines were subjected to molecular analysis to estimate the genome recovery rate (or conversion rate) of the BRS Esmeralda cultivar (recurrent parent) at the Plant Genetics Laboratory of Embrapa Genetic Resources and Biotechnology, Brasilia/DF. For this, 600 (six hundred) individual plants of each line were initially subjected to treatment with imidazolinone in a greenhouse according to the protocol previously described (Rangel et al. 2010), which was also used in the development of the irrigated rice cultivar BRS A706 CL (Rangel et al. 2022). In each line, 100 (one hundred) herbicide-resistant plants were selected for molecular analysis. The plants were transplanted into pots (one plant per pot) and samples of tender leaves from each plant were collected two weeks after transplanting and used for DNA extraction using the modified CTAB method (Ferreira and Grattapaglia 1996). Seeds were obtained from each plant, which were harvested and stored separately for future use. The DNA extracted was genotyped with a panel of SNP markers (Single Nucleotide Polymorphism) via the Sequenom MassArray platform, which uses a flexible SNP marker genotyping method based on the mass of alleles amplified by primer extension via polymerase chain reaction (www.sequenom.com). Allele separation by mass determination was performed using spectrometry (MALDI-TOF). A panel of 247 SNP markers, which are distributed across the 12 chromosomes of rice, was used to estimate the conversion rate on each chromosome, aiming to identify plants with the highest recovery rate of the recurrent parental genome. The ten plants with the highest genome recovery rate (≥98%) from each line and identical genetic profile were selected. The seeds collected from each of these plants were then sown separately in trays and 10 days after seedling emergence, the herbicide Kifix® was applied, at a dosage equivalent to 300 g ha^{-1}, plus Dash at a dose of 0.5 % v/v, to confirm resistance to IMI herbicides. Ten days after applying the herbicide, plants that did not present foliar damage, injury or death as a result of the herbicide were transplanted to the field, one plant per hole, in plots of ten 3 m long rows. Seeds were collected from plants of each line to obtain breeder´s seed and, later, genetic seed. Genetic seed multiplication for cultivar release was carried out after selecting the best family of the two lines based on the results of regional and Value of Cultivation and Use (VCU) trials.

As mentioned above, based on the results of herbicide resistance trials and evaluations of agronomic traits in the field, two lines (AB162666-RH and AB162664-RH) out of six were evaluated in the 2016/2017 and 2017/2018 seasons in 50 VCU trials distributed in 27 locations: Goiás (3 locations), Mato Grosso (5 locations), Maranhão (3 locations), Piauí (1 location), Acre (2 locations), Amazonas (1 location), Pará (4 locations), Rondônia (3 locations), Tocantins (3 locations), Roraima (2 locations). The AB162664-RH line stood out in the trials, presenting several favorable agronomic traits such as: high yield potential, grains with excellent industrial and culinary qualities and tolerance to the Kifix herbicide from the imidazolinone chemical group. Among the families of the AB162664-RH line evaluated for resistance after genotyping and with a higher conversion rate (98%) to the recurrent parental genome (BRS Esmeralda) based on DNA analysis, the AB162664-RH-83 family was selected to obtain 13 kg of high genetic purity breeder's seeds.

Additionally, to comply with the provisions of Article 22 and its sole paragraph of Law 9,456 of April 25, 1997, the AB162664-RH line underwent two years of Distinguishability, Homogeneity and Stability (DHE) tests conducted at the Capivara Farm Experimental Station, Embrapa Arroz e Feijão, Santo Antônio de Goiás/GO.

The AB162664-RH line was commercially released for planting in the Clearfield Rice Production System of Upland

Rice under the name BRS A504 CL and is recommended for cultivation in the North Central-West, Mid-North of the Northeast and North of Brazil.

PERFORMANCE CHARACTERISTICS

BRS A504 CL was evaluated during two seasons in the Embrapa's national network of VCU trials, conducted in partnership with state and municipal institutions, in states of the Central-West region (Goiás and Mato Grosso), Northeast region (Maranhão and Piauí) and the North region (Acre, Amazonas, Pará, Rondônia, Roraima and Tocantins). The traits grain yield (Figure 2), days to flowering and plant height were evaluated in most trials, while observations of lodging and disease severity were recorded in places where environmental conditions favored their occurrence (Table 1).

BRS A504 CL has a medium life cycle, with around 106 days from plant emergence to grain maturation, varying from 98 days in low latitude regions, such as Maranhão, to around 120 days in higher latitudes, such as Goiás. Cycle variations may occur depending on management conditions and cultivation environment. Flowering occurs on average 74 days after plant emergence. The average plant height is 104 cm, 5 cm lower than the control cultivars evaluated. Another interesting trait of the cultivar is "stay green", characterized by the persistence of the green color of the leaves or late senescence in the grain maturation phase, which contributes to its moderate tolerance to lodging, with a grade lower than the cultivar BRS A501 CL and similar to the cultivars BRS Esmeralda and AN Cambará (Table 1).

In VCU trials, the new cultivar had a high yield potential of up to 8,188 kg ha^{-1} (observed in the 2016/17 season, in São Mateus, Maranhão). Considering the average estimates from the joint analysis of all tests conducted, the average grain yield of BRS A504 CL was around 10.5% lower than that of the control "BRS Esmeralda" but statistically similar, according to the Tukey test at 5% of probability, to the cultivars BRS A501 CL (3,994 kg ha^{-1}) and AN Cambará (3,739 kg ha^{-1}). When considering the average yield by cultivation region, it was observed that in the Central-West region, BRS A504 CL is less productive than BRS Esmeralda (<13%), but similar to the other controls. In the Mid-North regions of the Northeast and North, the behavior of the new cultivar in comparison to the controls is, however, similar (Figure 2).

BRS A504 CL is resistant to herbicides from the imidazolinones chemical group. Imidazolinones control dicots, grasses and sedges. Imidazolinones inhibits the enzyme ALS (acetolactate synthase), also known as AHAS (acetohydroxyacid synthase). This enzyme processes a fundamental step in the biosynthesis of branched-chain amino acids such as valine,

Table 1. Average data for the BRS A504 CL cultivar and control cultivars in the Value of Cultivation and Use (VCU) trials for the 2016/17 (25 locations) and 2017/18 (25 locations) seasons, conducted in the states of Acre, Amazonas, Goiás, Maranhão, Mato Grosso, Pará, Piauí, Rondônia, Roraima and Tocantins

Agronomic Traits	BRS A504 CL	BRS A501 CL	BRS Esmeralda	AN Cambará
Days to flowering (days)	74	75	74	74
Plant height (cm)	104	111	109	107
Plant lodging[1]	1.40	2.10	1.70	1.60
Rice blast (leaf)[2]	1.60	1.70	1.70	1.90
Rice blast (neck)[2]	2.20	2.20	2.10	3.00
Rice brown spot[2]	2.70	2.60	2.50	2.50
Rice scald[2]	2.60	3.00	2.70	3.00
Rice grain spot[2]	2.40	2.40	2.20	2.40
National blast nursery scores[3]	3.30	2.50	3.20	3.60
Grain quality traits				
Whole grains (%)	65	-	62	66
Grain length (cm)	6.78	-	6.76	6.42
Grain width (cm)	1.87	-	1.88	1.82
Grain length/width ratio	3.62	-	3.60	3.53
Grain chalkiness area (%)	2.87	-	3.09	0.68
Grains w/chalkiness (%)	11.80	-	13.30	7.90

[1] Plant lodging = scores from 1 "all erect plants" to 9 "all lodging plants"). [2] Rice blast (leaf), Rice blast (neck), Rice brown spot, Rice scald and Rice grain spot: scores from 1 "very low sensitivity" to 9 "very high"). [3] NBN = Average scores for foliar blast assessments at the National Blast Nursery.

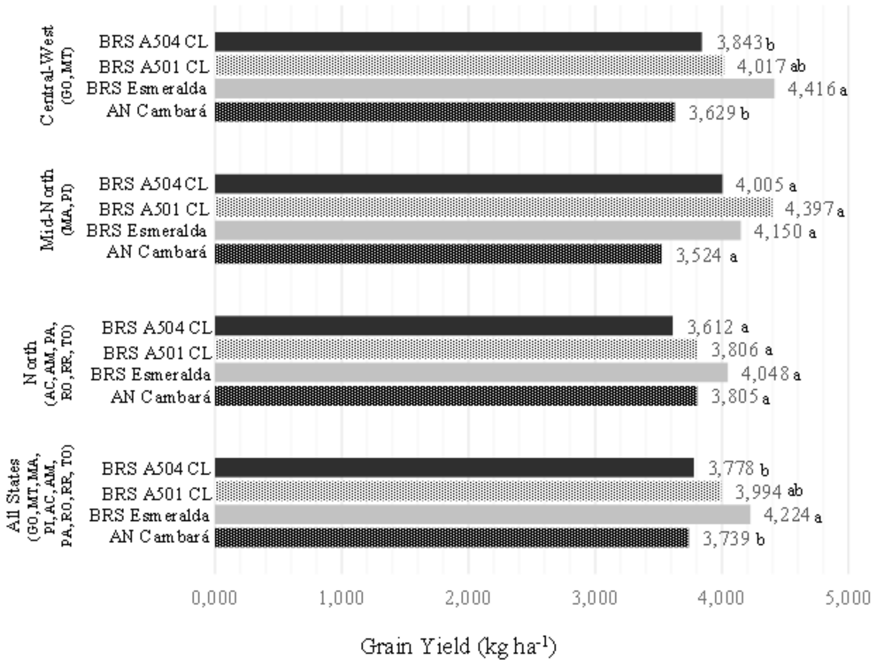

Figure 2. Average grain yield (kg ha^{-1}) of BRS A504 CL and control cultivars, in the Value of Cultivation and Use (VCU) trials of the 2016/17 seasons (25 locations) and 2017/18 (25 locations) conducted in the states of Acre (AC), Amazonas (AM), Goiás (GO), Maranhão (MA), Mato Grosso (MT), Pará (PA), Piauí (PI), Rondônia (RO), Roraima (RR) and Tocantins (TO). Within each grouping, means followed by the same letter do not present significant differences using the Tukey test, at 5% probability of error.

leucine and isoleucine. Deficiency of these essential amino acids causes plant death. These are herbicides that have very favorable attributes for use in agriculture, as they are efficient in controlling a large group of weeds, effective in low volumes, have low toxicity for mammals and are highly selective. The herbicide can be applied pre- and post-emergence in a conventional rotation system with other herbicides to reduce the appearance of resistant weeds.

Regarding resistance to diseases recorded in VCU trials, BRS A504 CL had a performance similar to BRS Esmeralda. The cultivar also participated in resistance assessments to leaf blast (*Magnaporthe oryzae*) at the National Blast Nursery (VNB – *Viveiro Nacional de Brusone*), conducted in a network by phytopathologists who are members of public rice breeding institutions in Brazil, in the 2016/2017 and 2017/2018 seasons. Considering the two seasons, in 74% of the evaluations, BRS A504 CL presented scores equal to or lower than 4 (Table 1). These results highlight the need to apply fungicides as a complementary disease control strategy, protecting the yield potential of the cultivar. Considering the crop's history, climatic conditions, planting time and management, it is recommended to carry out three preventive sprayings with fungicides, one in the vegetative phase and two in the reproductive phase, applying the first at the end of the heading stage, before the emission of the panicles, and the second approximately seven to ten days after the emission of the panicles, depending on the fungicide used. Preventive spraying prevents the establishment of epidemics in crops and contributes to the durability of resistance to *Magnaporthe oryzae* in commercial cultivars.

The industrial and culinary quality of the grains are fundamental traits that must be considered when releasing a rice cultivar. Grain quality analysis carried out at Embrapa Rice and Beans during the development stages of the BRS A504 CL cultivar revealed that the processed grains are of the long thin class, which is the one preferred by the Brazilian market. The grains have an average length (L) of 6.78 mm, 1.87 mm width (W) and a L/W ratio of 3.62. It is characterized by high translucency and whiteness, due to the low total chalkiness area of the grain mass (11.8%) (Table 1), while total chalkiness area of BRS Esmeralda grains was 13,3% and of AN Cambará was 7,9%. An S21 Rice Statistical Analyzer scanner (https://www.s21solutions.com) was used to obtain the grain measurements.

BRS A504 CL grains also have an intermediate amylose content (20.3%) and an intermediate gelatinization temperature (4.3), which are positive traits for the Brazilian market and related to good culinary attributes. In this regard, the BRS A504 CL has the grain quality desired by the Brazilian consumer, with grains that remain loose and soft after cooking, what was confirmed by laboratory cooking tests at Embrapa Rice and Beans carried out to simulate home cooking through the sensorial analysis of the texture of cooked rice.

A trait of great importance for the farmer and industry is the milling quality and whole grain percentage. The percentage of whole grains is directly related to the commercial value of rice. In the milling yield trials, BRS A504 CL showed a high average yield of whole grains (65%), thus being superior to the cultivar BRS Esmeralda and very similar to AN Cambará (Table 1).

In other trials carried out in the 2021/2022 season, in Santo Antônio de Goiás/GO, Sinop/MT and Vilhena/RO, five grain sample harvests were carried out for each cultivar, at 25, 32, 39, 46 and 53 days after flowering, determining grain moisture and whole grain yield. The results showed that BRS A504 CL seems to have maximum potential for whole grain yield when harvested 29 to 30 days after flowering. In harvests carried out after 35 days, BRS A504 CL maintains a higher yield of whole grains than the control cultivars BRS Esmeralda and AN Cambará.

It should be noticed that the leaves of BRS A504 CL remain green even during grain maturation stage (stay green trait). The advantages of the rice plant remaining green longer in the cycle include: (a) potential translocation of carbohydrates to the grains for a longer period of time due to continuous photosynthesis and, consequently, increased yield at the end of the cycle; (b) resistance to lodging due to stem stiffening caused to persistent photosynthetic activity.

FOUNDATION SEED PRODUCTION

BRS A504 CL is registered under number 47587, in the National Cultivar Registry (RNC) of the Ministry of Agriculture, Livestock and Supply (MAPA) of Brazil; and protected under number 20210179 in the National Cultivar Protection Service (SNPC) of MAPA. Information for cultivation of BRS A504 CL in the different Brazilian States can be obtained from MAPA's CultivarWeb system.

The breeder's seed was multiplied by Embrapa at the Experimental Field of Fazenda Palmital in Goianira/GO to obtain the genetic seed that was distributed to the partners of the upland rice improvement program, responsible for producing the basic seed.

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