BRS AG: first cultivar of irrigated rice used for alcohol production or animal feed

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Abstract: BRS AG is an irrigated rice cultivar developed by Embrapa, recommended for cultivation in the state of Rio Grande do Sul. It is intended for grain alcohol production or animal feed, with average thousand-grain weight of 52g (double of the conventional cultivars) and average yield of 8193 kg ha⁻¹.

Key words: Oryza sativa L., large grain, crop diversification.

INTRODUCTION

The economy in the southern half of Brazil is mainly based on agriculture, the irrigated rice and the production of beef and sheep meat being the most prominent sectors. The soybean, maize and sorghum cultures, which are naturally the option to be part of the production system of these areas, though present some problems. The pointed reasons are the climate featuring water restriction in part of the territory, the difficulties of farming in lowlands (hydromorphic, poorly drained soils - ideal for the cultivation of irrigated rice, but challenging for other crops). From the rice production perspective, the RS state meets more than 65% of the Brazilian demand, with approximately 1.1 million hectares being cultivated per year (CONAB 2016).

With the advent of plant breeding, the rate of the genetic progress of rice varieties was intensively accelerated (Breseguello and Coelho 2013), resulting in significant increases in the yield potential.

Thus, Brazil has achieved self-sufficiency in rice production and it seems inevitable that it will continue in this condition of increased production (Adami and Miranda 2011), so there is the need to develop strategies for diversification and development of new ways in the paddy chain. Consequently, thereby avoiding that the surplus production generated reduces the price paid for the product, which would make the activity economically unviable, not covering the production costs.

In this context of the productive chain and the search for renewable energies that are economically viable, BRS AG was developed to use of the rice as a raw material for the production of cereal alcohol or animal feed. It is worth emphasizing that the RS state imports 98% ethanol for consumption with a higher spending to R$ 1 billion per year. Therefore, the aim of this article is to describe agronomically the first cultivar of irrigated rice developed by Embrapa for this purpose.

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PEDIGREE AND BREEDING METHOD

The BRS AG is the result of a simple cross between the American irrigated rice cultivar Gulfmont and the SLG1 lineage, of Japanese origin, held in 1994/1995. The F1 seeds were harvested and stored for planting in the 1995/1996 crop, which were sown in a greenhouse and, after reaching 20 cm in height, were transplanted to the experimental field of Embrapa Temperate Climate Lowlands Station. F1 lines were transplanted alongside their parents for comparison and roguing of selfing. After the rouguing, immature anthers were collected from the effectively hybrid plants that were placed to callus induction “in vitro” in a laboratory and subsequent regeneration of homozygous plants, being acclimatized in a greenhouse for seed production. The chromosome duplication process occurred spontaneously without use of colchicine.

In the 1996/1997 crop, the homozygous seeds were sown on the field originating the CL 485 lineage, where it was observed the agronomic performance of the same. Among the assessed traits, the large size of the grains of the lineage stood out, with average thousand-grain weight around 52 g, almost the double of the mass belonging to the cultivars currently used for the production of irrigated rice in Brazil.

This lineage, by not following the standards required by the national preference, which is a grain of long, thin type, vitreous and loose after cooking, was kept in the program as a source of genetic variability.

In the 2010/11 crop, due to a production surplus in the rice production chain, Embrapa dealt with the demand of a differentiated pattern of rice, seeking to economically enable the paddy activity. In search of a cultivar that would be presented as an alternative of raw material for animal feed or for the production of grain alcohol, removing part of the long fine grain from the consumer market.

In order to satisfy this request, in the 2011/2012 crop, the CL 485 lineage was recovered, which was recoded as AB11047, being part of the trials of Value of Cultivation and Use (VCU), in different parts of Rio Grande do Sul. In 2011/2012, these trials were conducted in Capão do Leão, Santa Vitória do Palmar and Alegrete. In 2012/2013, the trials were conducted in Capão do Leão, Santa Vitória do Palmar and Capivari do Sul.

PERFORMANCE CHARACTERISTICS

The BRS AG is the result of a crossing performed at Embrapa Temperate Climate, which involved genes of the introduced genotype SLG1 (super large grain) (Takita 1983), whose grain dimensions are larger than those of the conventional rice, being nicknamed “Gigante” (Giant). It has an average thousand-grain weight equivalent to 52 g, while the majority of irrigated rice cultivars show lower values, as BRS Pampa, showing 25.6 g (Table 1).

The plants of this cultivar (Table 1) have a biological cycle around 126 days, from emergence to maturity. The average plant height is 110 cm, with distinctive architecture (modern-Philippine), however, the thickness of the stem is 5.5 mm, which gives them strong culms, resistant to lodging, despite this high stature of the plants.

The leaves are hairy, the flag leaf being classified as decumbent. It is abscission-resistant, therefore, without risk of becoming a weed plant in the paddy crop. Allied to this fact, the seeds of this lineage lose the germination capacity and the vigor with great ease, helping to reduce their infestation potential, thus they can not be compared to the main weed crop: the red rice.

Through the 35 morphological characters evaluated, it was found that the BRS AG had a high genetic distance from the modern-Philippine cultivars previously released by Embrapa, corroborating with Streck et al. (2017). Thus, this release does not follow mentioned by Rabelo et al. (2015) who found that the irrigated rice Brazilian cultivars have narrow genetic base. This result can be explained mainly by the intrinsic characteristics regarding the type of grain and plant architecture, the BRS AG neither has grains of the indica subspecies type nor modern-Philippine architecture. Yet the Arborio genotype, widely used in the Italian cuisine, showed the greatest genetic similarity (Figure 1), being considered the one with best agronomic comparison to the “Gigante”.

Regarding the genetic potential of productivity, the BRS AG cultivar stands out greatly in relation to the Arborio genotype, showing yield plateaus around 10,000 kg ha⁻¹. In comparative tests in the municipalities of Capão do Leão and Santa Vitória do Palmar (Figure 2), it showed an average yield of 8,193 kg ha⁻¹, approaching the average yield obtained by the BRS Pampa (9,215 kg ha⁻¹), which is one of the main cultivars in Rio Grande do Sul.
Notwithstanding, the BRS AG “Gigante”, by having very large grains and a low amylose-amylopectin ratio (starchy grains), results in a lower quality in cooking, not adapting to the standard Brazilian consumption. It is the first rice cultivar intended for uses other than human consumption, as grain alcohol production or animal feed.

To check the efficiency for these purposes, it were made assessments of the hydrolysis processes, the ethanol fermentation and the bromatological composition. These activities were carried out by the EMBRAPA Agroenergy

**Table 1.** Comparison of the grain and plant characteristics between the irrigated rice cultivars BRS AG and BRS Pampa

| Characteristics                  | BRS AG       | BRS PAMPA   |
|----------------------------------|--------------|-------------|
| **Grain**                        |              |             |
| Caryopsis shape*                 | half spindle-shaped | spindle-shaped |
| Awns                             | absent       | absent      |
| Glumes colour                    | straw        | straw       |
| Colour of apiculus in flowering  | white        | white       |
| Colour of apiculus in maturaty   | white        | white       |
| Pubescence grain                 | present      | present     |
| Length of grain (mm)**           | 7.82         | 9.82        |
| Width of grain (mm)**            | 3.64         | 2.20        |
| Thickness of grain (mm)**        | 2.60         | 2.00        |
| Length of caryopsis (mm)**       | 6.30         | 7.19        |
| Width of caryopsis (mm)**        | 2.60         | 1.96        |
| Thickness of caryopsis (mm)**    | 1.90         | 1.76        |
| Length / width \(\frac{\text{cm}}{\text{cm}}\) ratio** | 2.15         | 3.59        |
| 1000-grain weight (g)**          | 52.00        | 25.60       |
| Total income (%)**               | 77.60        | 68.00       |
| Intact grain (%)****             | 71.60        | 62.00       |
| Endosperm amylose content        | high         | high        |
| Gelatinization temperature       | low          | low         |
| Potential productivity (t ha\(^{-1}\))*** | 10           | 10          |
| **Plant**                        |              |             |
| Plant type                       | intermediate | modern      |
| Days to flowering                | 96           | 88          |
| Days to maturity**               | 126 (medium) | 118 (precocious) |
| Plant height (cm)**              | 110          | 96          |
| Culm length (cm)**               | 88           | 72          |
| Panicle length (cm)**            | 23           | 24          |
| Panicle exsertion*               | medium       | medium      |
| Leaf colour                      | green        | green       |
| Flag leaf angle                  | decumbent    | erect       |
| Auricle colour                   | light green  | light green |
| Ligule colour                    | colorless    | colorless   |
| Culm internode color             | light green  | light green |
| Anthocyanin colouration on the culms | absent      | absent      |
| Panicle type                     | intermediate | intermediate |
| Leaf blade pubescence            | present      | present     |
| Dehiscence*                      | tolerant     | intermediate |
| Lodging tolerance*               | tolerant     | tolerant    |
| Tilling*                         | low          | high        |
| Iron indirectly toxicity**       | moderate tolerance | moderate tolerance |
| Rice blast in leaf and panicle** | moderate resistant | moderate resistant |
| Stain grains**                   | moderate sensitive | moderate resistant |

*Length / width \(\frac{\text{cm}}{\text{cm}}\) ratio

** Can be changed depending on the characteristics of the cultivation environment.

*** Grains in shell, 13% humidity, observed in experiments conducted by Embrapa.

**** The BRS AG cultivar underwent no polishing, while the BRS Pampa was peeled and polished in a Suzuki test device.
The actual yield of the enzymatic hydrolysis of the BRS Pampa and BRS AG grains in the enzyme concentration of 0.1% (m v\(^{-1}\)), by the action of the alpha-amylase (dextrinizing) and amyloglucosidase (saccharification) enzymes, was approximately 108.9 g L\(^{-1}\) glucose for both genotypes after 24 hours of the procedure. Yet in the enzyme concentration of 0.2%, the yield was 132 g L\(^{-1}\) for the BRS Pampa and 155.1 g L\(^{-1}\) for the BRS AG. Thus, the efficiencies of the enzymatic hydrolysis for the ground grains of the BRS Pampa and BRS AG cultivars were approximately 89.4% and 104.7%, respectively, for the enzyme concentration of 0.2% (Table 2).

After 24 hours of fermentation for the hydrolysate (with cake) with 0.2% enzyme concentration, it were verified yields of 3.57 g (L h)\(^{-1}\) for BRS Pampa and 4.04 g (L h)\(^{-1}\) for BRS AG. The ethanol productivity for testing the hydrolysates without the presence of the cake, arising from the process with 0.2% enzyme, showed results of 4.92 and 4.53 g (L h)\(^{-1}\) for BRS Pampa and BRS AG, respectively (Table 3).

The bromatological analyses carried out (ground grains) and those regarding the products from the hydrolysis and fermentation processes (Table 4) showed high content of dry matter, with values over 90% for all samples and values less than 6% for the content of mineral matter (ash). The Acid Detergent Fiber (ADF) results were below 16% for most of the samples, while the Neutral Detergent Fiber (NDF) values ranged from 7 to 28.5%, highlighting the sample of the BRS AG rice, analyzed together with the yeast, which showed a percentage of 41.03%. The lignin and ether extract contents were below 8% for most of the analyzed material.

All samples showed a high crude protein content, with values above 30% for most of these materials. The products from fermentation processes, with the presence of yeast, had significant values for total protein, in which the material

**Table 2.** Efficiency of the enzymatic hydrolysis (%) of samples of the irrigated rice cultivars BRS AG “Gigante” and BRS Pampa, by the alpha-amylase (90 °C for one hour) and amyloglucosidase (60 °C for 6 hours) enzymes, in the concentration of 0.2% (m v\(^{-1}\)).

| Cultivar  | Sample (g) | Volume reactor (L) | Hydrolysis yield (g L\(^{-1}\)) | Enzymatic efficiency (%) |
|-----------|------------|--------------------|-------------------------------|--------------------------|
| BRS Pampa | 200        | 1.2                | 132.4                         | 89.4                     |
| BRS AG    | 200        | 1.2                | 155.1                         | 104.7                    |

Figure 1. Dendrogram resulting from the genetic distance analysis of 10 irrigated rice genotypes characterized by 35 morphological characters, obtained by the UPGMA grouping method.

Figure 2. Average yield of grains of the irrigated rice cultivar BRS AG in relation to BRS Pampa and Arborio in the municipalities of Capão do Leão and Santa Vitória do Palmar in the 2011/2012 and 2012/2013 crops.
In this context, this type of grain of the BRS AG cultivar, referred to as “DHC” (UHC - unfit for human consumption), has high potential as a feedstock for grain alcohol production and for animal feed. Another advantage of the BRS AG concerns the shell/grain ratio, when compared to conventional grain cultivars, of long, thin type, presenting 17.5% shell, while the conventional cultivars present 22%.

**BASIC SEED PRODUCTION**

The BRS AG is registered in the National Register of Cultivars (RNC) and protected by the Ministry of Agriculture, Livestock and Supply (MAPA). The Business Office of Capão do Leão, of Embrapa Products and Market is responsible for providing the basic seeds of the aforementioned cultivar.

It is indicated the sowing of the cultivar BRS AG “Gigante”, following the agricultural zoning for the irrigated rice crop in Rio Grande do Sul. It is recommended that the sowing occurs respecting the cycle of the cultivar in interaction with the cultivation environment, so that the differentiation of primordia occurs until January 1 or as close to that date as possible.

The density of suitable seeds (100% germination capacity) should be about 80 seeds per linear meter (about 200 kg ha⁻¹) for the on-line system, since it does not show high tillering capacity, ensuring a plant population from 200 to 300 plants per square meter (SOSBAI 2014).

The BRS AG shows positive response to different levels of basic and coverage fertilization, without the lodging of plants.

The harvest of this cultivar, aiming to minimize the natural abscission and prevent the grain breakage during the manufacturing process, must be performed when the grain moisture content is between 23% and 18%.

In the homogeneity tests, BRS AG “Gigante” has shown to be uniform, without the presence of atypical plants, demonstrating to be genetically stable, even by the fact that the homozygosity was obtained through the culture of immature anthers.

**Table 3.** Productivity, substrate yield and efficiency of fermentation of the hydrolysates of samples of the irrigated rice cultivars BRS AG “Gigante” and BRS Pampa, with and without the presence of the residual cake (enzymatic post-hydrolysis, for 24 hours, in the concentration of 0.2%)

| Cultivar | Fermentation conditions | Ethanol productivity (L h⁻¹) | Substrate yield (gg⁻¹) |
|----------|-------------------------|-----------------------------|------------------------|
| BRS Pampa | With residual cake | 3.57 | 0.63 |
|           | No residual cake       | 4.92 | 0.89 |
| BRS AG   | With residual cake     | 4.04 | 0.83 |
|           | No residual cake       | 4.53 | 0.79 |

**Table 4.** Bromatological analyses of the cakes of the irrigated rice cultivars BRS AG “Gigante” and BRS Pampa, enzymatic post-hydrolysis (without yeast) and post-hydrolysis/fermentation (with yeast), in natural or raw state (ground grain). Analyses made in experiments with the enzyme concentration of 0.2% (v v⁻¹)

| Cultivar | Sample          | Dry matter | Ash | Neutral detergent fiber | Acid detergent fiber | Lignin | Ether extract | Protein         |
|----------|-----------------|------------|-----|-------------------------|---------------------|--------|---------------|-----------------|
| BRS Pampa| Gross           | 90.06 ± 0.32 | 3.81 ± 0.05 | 8.22 ± 0.32 | 3.57 ± 0.187 | 0    | 4.60 ± 0.352 | 12.22 ± 0.126 |
|         | Yeast extract   | 93.82 ± 1.04 | 4.27 ± 0.73 | 0.94 ± 0.26 | 0.56 ± 0.012 | 0    | 1.64 ± 0.12  | 38.28 ± 0.067 |
|         | Cakeno yeast    | 93.64 ± 0.10 | 1.64 ± 0.94 | 18.96 ± 0.23 | 11.01 ± 0.095 | 7.60 ± 0.384 | 5.76 ± 0.18 | 31.42 ± 0.365 |
|         | Cake with yeast | 96.00 ± 0.27 | 2.30 ± 0.04 | 21.26 ± 2.17 | 9.99 ± 0.926 | 7.33 ± 2.58 | 3.05 ± 0.31 | 46.45 ± 0.157 |
| BRS AG  | Gross           | 91.26 ± 0.44 | 0.71 ± 0.26 | 7.28 ± 0.27 | 2.96 ± 0.122 | 1.31 ± 0.312 | 2.08 ± 0.14 | 11.99 ± 0.343 |
|         | Yeast extract   | 94.21 ± 0.11 | 5.80 ± 1.42 | 0.57 ± 0.17 | 0.22 ± 0.093 | 0    | 1.67 ± 0.29 | 39.89 ± 0.085 |
|         | Cakeno yeast    | 92.64 ± 0.15 | 0.27 ± 0.05 | 28.46 ± 0.44 | 15.31 ± 0.384 | 5.17 ± 1.04 | 6.43 ± 0.32 | 34.44 ± 0.130 |
|         | Cake with yeast | 95.79 ± 0.20 | 3.41 ± 0.01 | 41.03 ± 0.22 | 14.78 ± 0.228 | 2.20 ± 2.34 | 6.77 ± 0.10 | 51.54 ± 0.193 |

from the BRS AG cultivar reached the percentage of 51.54%, against 46.45% of the BRS Pampa cultivar. Kunrath et al. (2010) studied, with the same analytical technique, the chemical composition of defatted rice bran for pigs and found values similar to those of this work, showing great potential of the BRS AG cultivar for animal nutrition.
REFERENCES

Adami ACO and Miranda SHG (2011) Transmissão de preços e cointegração no mercado brasileiro de arroz. Revista de Economia e Sociologia Rural 49: 55-80.

Breseguello F and Coelho ASG (2013) Traditional and modern plant breeding methods with examples in rice (Oryza sativa L.). Journal of Agricultural and Food Chemistry 61: 8277-8286.

CONAB - Companhia Nacional de Abastecimento (2016) Acompanhamento da safra brasileira: Grãos. Available at <http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_06_09_16_49_15_boletim_graos_junho__2016_-_final.pdf>. Accessed on June 21, 2016.

Kunrath MA, Kessler AM, Ribeiro AML, Vieira MM, Silva GL and Peixoto FD'Á (2010) Metodologias de avaliação do valor nutricional do farelo de arroz desengordurado para suínos. Pesquisa Agropecuária Brasileira 45: 1172-1179.

Rabelo HO, Guimarães JFR, Pinheiro JB and Silva EF (2015) Genetic base of Brazilian irrigated rice cultivars. Crop Breeding and Applied Biotechnology 15: 146-153.

SOSBAI - Sociedade Sul-brasileira de Arroz Irrigado (2014) Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. Editora SOSBAI, Bento Gonçalves, 189p.

Streck EA, Aguiar GA, Magalhães Júnior AM, Facchinello PHK and Oliveira AC (2017) Variabilidade fenotípica de genótipos de arroz irrigado via análise multivariada. Revista Ciência Agronômica 48: 101-109.

Takita T (1983) Breeding of a rice line with extraordinarily large grains as a genetic source for high yielding varieties. Japan Agricultural Research Quarterly 17: 93-97.