Estimates of genetic selection gain in irrigated F₃ rice families

Estimativa do ganho de seleção em famílias F₃ de arroz irrigado

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Abstract

Rice production estimate in Brazil is 10.7 million tons, of which approximately 80% comes from the flood irrigation system in the southern region. Rice breeding programs have an important role in the development of cultivars with high production potential and industrial quality. This study estimates variance components and genetic parameters in F₃ families, and predicts the genetic gain in rice grain quality for the F₄ generation. We used fifty-six (56) F₃ families obtained from the advance of generations of the hybrid BRSCIRAD 302. This hybrid was selected for its traits of agronomic and scientific interest, such as grain yield and industrial yield. The hybrid was developed by Embrapa in partnership with CIRAD (Center for International Cooperation in Agronomic Research for Development) for Rio Grande do Sul State through the crossing of CIRAD 464 (M) × SBT 106 (F). The quality of the selected families was analyzed for amylose content, white belly, and gelatinization temperature. The F₃ families showed low gelatinization temperature. Selection of BRSCIRAD 302 hybrid families with smaller white belly spots and higher amylose content may be more successful if carried out between F₃ families. Twenty-two (22) families showed amylose content higher than that of the highest parent (29.44%). The predicted genetic gain in amylose content was 4.85% for families derived from the hybrid BRSCIRAD 302.

Additional keywords: genetic variation; grain quality; Oryza sativa L.

Introduction

The rice crop has great social and economic importance and is considered a crop that can alleviate world hunger. It is the third most produced cereal in the world, being the main component of the basic food basket for more than half the population (Magalhães Júnior & Oliveira, 2008; Fonseca et al., 2018a; Streck et al., 2018a).

Rice is one of the most consumed foods in Brazil, being characterized within the Brazilian economy as a product of very inelastic demand, that is, its demand does not change substantially with the increase in price. Regarding rice cultivation in Brazil, especially in irrigated crops in Rio Grande do Sul State, the quality and quantity of the product are often impaired by abiotic stresses such as low temperatures (Fonseca et al., 2018b).
Quantity and quality are important aspects for all the agents involved in the production, commercialization, and consumption of the cereal. However, genetic breeders should focus on consumer demands, which are made possible by the producers and social agents linked to the processing facilities. The analysis of the genetic breeding program for irrigated rice at Embrapa Clima Temperado - Estação Terras Baixas showed significant annual genetic progress between 1972 and 2016 for the main attributes of grain quality. The program has provided cultivars that meet the national demand for grain quality in the various segments of the rice production chain (Streck et al., 2018a). In this sector, genetic breeding has successfully achieved desirable production traits and industrial quality (Streck et al., 2018b).

From a genetic point of view, the breeding and evolution of cultivated plants have many characteristics in common: they require genetic variation, a selection method, and a degree or form of isolation to preserve the traits that arise (Briggs & Knowles, 1999). Therefore, identification of variation sources, which is the basis of plant breeding (Streck et al., 2017), is of fundamental importance for progress in improving agronomic traits of interest.

The concept of quality is conceived and viewed differently depending on the purpose of consumption, the ethnic group involved, postharvest processing, among others (Xu et al., 2015). The demands of all segments of the market chain must be considered in determining the quality of a cultivar. Thus, it is important to know the organoleptic and physicochemical characteristics of rice grain, its cooking and appearance properties, which define quality standards in the market.

Grain quality traits limit commercialization in consumer countries. Physical appearance, culinary and sensory properties, and nutritional value are fundamental in the adoption of new varieties. The classification of each grain quality parameter varies according to the culture and consumption habits (Magalhães Júnior et al., 2011). To meet market demand and requirements for quality standards, the main rice research institutions have been developing rice cultivars with high yield and high grain quality, a fact that can be seen in the genetic gain in grain quality in the study of Streck et al. (2018b).

Thus, this study estimates variance components and genetic parameters in F3 families, and estimate the genetic selection gain in rice grain quality for the F3 generation.

**Material and methods**

**Plant Material**

The present study used F3 progenies from the advance of generations of the hybrid BRSCIRAD 302 (F1). This hybrid comes from the crossing between CIRAD 464 (M) × SBT 106 (F1), and was developed in a collaborative process between Embrapa (Temperate Climate) and CIRAD for the conditions of Rio Grande do Sul State. The superiority of grain yield (10.2 t ha⁻¹), combined with wide adaptability and stability and high industrial performance, were fundamental traits for its selection in this study (Streck et al., 2018c).

**Generation Advance**

In the 2011/2012 crop year, 4,500 seeds of the rice hybrid (F2) were sown in two seasons and two locations: an experimental field at Estação Terras Baixas, belonging to Embrapa Clima Temperado, in Capão do Leão city, Rio Grande do Sul State; and an experimental field of IRGA (Instituto Rio Grandense do Arroz), in Cachoeirinha city, Rio Grande do Sul State. One hundred (100) plants were selected from each season and location, totaling 400 plants (F3) seeds. More details are obtained in Fonseca et al. (2019).

**Conduction of F3**

Seeds of 400 F3 plants were sown in the 2012/2013 crop year in the experimental field of Embrapa Clima Temperado - Estação Terras Baixas. Sowing was performed on December 17 and seedlings germinated on December 28, 2012. The area was managed according to the technical recommendations for irrigated rice cultivation (Sosba, 2014). One hundred and fifty-four (154) plants were selected from fifty-six (56) families. Plants were selected through visual observation, considering agronomic traits of interest such as: lodging resistance, plant architecture, plant height, panicle exertion, yield capacity, grain spot, grain size, spikelet sterility, tilling capacity, disease resistance.

**Experimental Design**

The experimental design used in the F3 generation was that of families with interspersed parents. 56 families were used, where the parents were arranged in three interspersed replicates between the families. Six parent plants were evaluated within each replicate.

**Evaluated Traits**

The seeds of the selected plants were subjected to the following quality analyses: amylose content, white belly, and gelatinization temperature.

Amylose content analysis: amylose is the linear starch fraction found in nonglutinous cultivars. Amylose content was analyzed according to the methodology described by Ciat (Martínez, 1989), using 90 whole, polished, and healthy grains. The content is thus framed by the following criteria: sticky - 0 to 2% amylose; glutinous - 3 to 10% amylose; low - 11% to 22% amylose; intermediate - 23% to 27% amylose; and high - 28% to 32% amylose. Data were expressed in percentages (%).

Amylose content was measured using the iodine colorimetric method. After making a flour from the rice grains, 100 mg of sample was weighed and trans-
ferred to a volumetric flask. Then, 1 mL of 95% alcohol was added to the flask, with care to dilute the rice flour for avoiding lump formation. Subsequently, 9 mL of sodium was added to the solution, which was heated for 9 minutes in a water bath (100 °C) to gelatinize the starch.

After cooling for 30 minutes, the flask walls were washed with distilled water, making up the total volume, and the solution was stirred. From this solution, a 5 mL aliquot was placed in a 100 mL flask, to which 1 mL of 1N acetic acid and 2 mL of iodine solution were added. Then, the total volume was filled with distilled water, and the solution was stirred and left to rest for thirty minutes.

The iodine solution was prepared by dissolving 0.2 g of metal iodine and 2 g of potassium iodide in 100 mL of distilled water. The absorption of the solution was measured using a spectrophotometer. Amylose content was quantified from a standard curve generated by absorption values of three known rice cultivars: IRGA 417, Bluebelle, and Bico Torto, with high, intermediate, and low amylose content, respectively.

White belly rate was determined in samples of whole grains after breeding, with scores from 0 to 5, where Wb= √(x + 0.5) (Martinez, 1989). White belly rate data were transformed following: Wb=Vx + 0.5, where Wb: white belly of the transformed data, and x: the value of the collected datum.

Gelatinization temperature: the gelatinization temperature of starch refers to the cooking temperature at which the water is absorbed and the starch granules increase irreversibly in size, with simultaneous loss of crystallinity. It is analyzed according to the methodology described by Ciat (Martinez, 1989). White belly rate data were transformed following: Wb=Vx + 0.5, where Wb: white belly of the transformed data, and x: the value of the collected datum.

Statistical Analysis
Data were submitted to analysis of variance according to the experimental design. In this sequence, sums of squares (SS) and mean squares (MS) were obtained for each of the model components. Subsequently, variance components and genetic parameters were estimated for the variables amylose content and white belly. Finally, the selection differential (SD) and genetic gain (GG) were estimated with the inbreeding coefficient (F) set at 75%, since the grains harvested from F2 plants were in the F3 generation. The coefficient was obtained according to Falconer & Mackay (1996), considering the self-fertilization mating system.

\[ F_t = \frac{1}{2} \left(1 + F_{t-1}\right) \]

Wherein: F is the inbreeding coefficient and, t corresponds to the generation.

Since the families were evaluated in trials without replicates, it was necessary to correct the data according to the environmental effects estimated from parental information, as indicated by Cruz et al. (2012).

\[ V_{cx} = V_{ox} - \epsilon_i + \frac{p_i - p_f}{p_i - p_f} (\epsilon_f - \epsilon_i) \]

Wherein:
- \( V_{cx} \): Corrected value of the segregating line in row x;
- \( V_{ox} \): Original value of the segregating line in row x;
- \( \epsilon_i \): value of the environmental effect that occurs in parents who are in position i of the rows;
- \( p_i \): initial extreme position occupied by the parents;
- \( p_f \): final extreme position occupied by the parents;
- \( \epsilon_f \): position occupied by the segregating row, whose value is being corrected;
- \( \epsilon_i \): value of the environmental effect that occurs in parents who are in position i of the rows.

Variance components were estimated according to the following equations:

**Environmental variance between \((\hat{\sigma}^2_E) \) families:**

\[ \hat{\sigma}^2_E \text{ between} = \frac{1}{2} \left( \frac{\hat{\sigma}^2_E \text{ between } G1 + \hat{\sigma}^2_E \text{ between } G2}{n} \right) \]

\[ \hat{\sigma}^2_E \text{ within } = \frac{\hat{\sigma}^2_E \text{ within } G1 + \hat{\sigma}^2_E \text{ within } G2}{2} \]

Wherein:
- \( \hat{\sigma}^2_E \text{ within } G1 \) is the environmental variance of parent 1;
- \( \hat{\sigma}^2_E \text{ within } G2 \) is the environmental variance of parent 2;
- \( n \) is the number observations (plants) associated with each parent.

**Phenotypic variance between progenies \((\hat{\sigma}^2_P) \), within progenies \((\hat{\sigma}^2_P) \), and total phenotypic variance \((\hat{\sigma}^2_P) \):**

\[ \hat{\sigma}^2_P \text{ between } = \frac{\hat{\sigma}^2_P \text{ between progenies}}{n} \]

\[ \hat{\sigma}^2_P \text{ within } = \hat{\sigma}^2_P \text{ within progenies} \]

\[ \hat{\sigma}^2_P \text{ total } = \hat{\sigma}^2_P \text{ within progenies } + \hat{\sigma}^2_P \text{ between progenies} \]

Genetic variance between \((\hat{\sigma}^2_G) \) and with within \((\hat{\sigma}^2_G) \) progenies:

\[ \hat{\sigma}^2_G \text{ between } = \hat{\sigma}^2_P \text{ between } - \hat{\sigma}^2_E \text{ between } \]

\[ \hat{\sigma}^2_G \text{ within } = \hat{\sigma}^2_P \text{ within } - \hat{\sigma}^2_E \text{ within } \]

Additive genetic variance \((\hat{\sigma}^2_A)\):

\[ \hat{\sigma}^2_A = \frac{\left( \hat{\sigma}^2_G \text{ between } - F \hat{\sigma}^2_G \text{ within } \right)}{F(1 + F)} \]
Table 1 - Summary of the analysis of variance, sum of square and mean square of parent 1, parent 2 and generation F3 for the variable white center.

| Source of variation | Parent 1 | Parent 2 |
|---------------------|----------|----------|
|                      | FD       | SS        | MS       | SS        | MS        |
| Between plots       | 2        | 0.001089  | 0.000544** | 0.0000    | 0.000000** |
| Within plots        | 6        | 0.009900  | 0.001650  | 0.009900  | 0.00165   |
| Total               | 8        | 0.010900  | 0.001363  | 0.009900  | 0.00124   |
| Variance component  | 0.000000  | 0.000000  |           |           |           |
| Between           |          |           |           |           |           |
| Generation F3      |          |           |           |           |           |

| Source of variation | FD       | SS        | MS       |
|---------------------|----------|----------|----------|
| Between plots       | 55       | 1.723087  | 0.031329** |
| Within plots        | 98       | 0.726900  | 0.007417  |
| Total               | 153      | 2.449300  | 0.016008  |
| Variance component  | 0.008699  |           |           |

FD: freedom degrees, SS: sum of squares, MS: medium square, **: no significant at 5% of error probability. ** and ***: significant at 5 and 1% of error probability by F test.
Analysis of variance for the variable amylose (Table 2) showed no significance between plots for both parents (1 and 2). On the other hand, the analysis between families showed a significant effect, which points to genetic variability between F₃ families.

Table 3 shows the estimates of the variance components and genetic parameters of the variables white belly and amylose content.

The phenotypic variance for the trait white belly is higher between families than within families. However, knowing the importance of phenotypic variance, the plant breeder is guided by the causes of genetic variations for selection purposes (Falconer & Mackay, 1996). According to Magalhães Júnior et al. (2011), recent changes in the classification of rice indicate that white-belly grains can be considered as plastered if grain opacity exceeds 50%, depreciating the product. Although white belly has several origins, such as early harvest and out-of-season heat waves, the genetic issue is present, and genotypes with a low incidence of white belly should be sought. Grain dimensions are also important, as the length/thickness ratio determines the rice class. The following classes are presented as a function of grain type: long-thin, long, medium, short, and mixed, among which the long-thin class stands out in the market.

Genetic variance between families for the traits white belly and amylose content was 0.011307 and 1.593316, respectively. Genetic variance within families was 0.005767 for white belly and 0.967149 for amylose content. As expected, for the two traits evaluated, estimates of genetic variance between families were higher than estimates within families. In addition to expressing genetic variation among individuals in a population, genotypic variance can be treated in the biometric context and be subdivided into causes of variation (additive, dominance, and epistatic). These are important components for the establishment of selection strategies in breeding programs.

Additive genetic variance was 0.005319 for white belly and 0.661298 for amylose (Table 3). This variance represents the inheritable fraction of the trait. Only the phenotypic value of an individual can be measured; however, it is the genetic value that will influence the next generation. Therefore, it is important to know how much of the phenotypic variation is attributed to the genotypic variation, and this is measured by heritability (Falconer & Mackay, 1996; Cruz, 2005).

Amylose is considered the main factor related to the sensory quality of rice. Grain amylose content can be classified as: reduced content, for grains with 10 to 20% amylose; medium content, from 20 to 25%; and high content, from 25 to 33%. Grains can also be grouped into waxy rice - with 0 to 2% amylose; Japanese rice (short grain) - 18 to 20%; Indian rice - 25 to 30%; three rice - 19 to 20%; basmati rice - 25 to 27%; and jasmine rice - 22 to 25% (Botelho et al., 2009).

Amylose content is related to the textural properties of rice, such as softness, cohesion, color, brightness, and expansion volume, providing information about the changes that occur during cooking. Rice with high amylose content usually presents dry and loose grains, which can harden after cooking. Low amylose cultivars have soft, watery, and sticky grains when cooking. Cultivars with intermediate content have dry, loose, and soft grains, even after cooling (Magalhães Júnior et al., 2011).

Dominance variation for the traits white belly and amylose was 0.017750 and 3.207298, respectively. The dominance variance in the traits revealed that the selection would be less effective in the initial generation, with high variance values. Dominance deviations are not likely to be maintained in advanced breeding generations.

Estimates of broad-sense heritability between families for the traits white belly and amylose were high: 0.992039 and 0.955575, respectively. For broad-sense heritability within families, these estimates were 0.777548 and 0.6885147, respectively. Estimates of broad-sense heritability between and within families were considerably high. However, these estimates may be inflated by dominance deviations, since all genetic variance (additive and dominance deviations) is considered. Heritability estimates express the confidence of the phenotypic value as a guide for the genotypic value, and are part of most of the expressions used in genetic breeding, mainly in the prediction of genetic gains from selection (Falconer & Mackay, 1996; Cruz, 2005).

Table 2 - Summary of the analysis of variance, sum of squares and mean square of parent 1, parent 2 and generation F₃, for the variable amylose.

| Source of variation | Parent 1 | Parent 2 | Generation F₃ |
|---------------------|----------|----------|----------------|
|                      | FD       | SS       | MS             | FD       | SS       | MS             |
| Between plots       | 2        | 0.666667 | 0.333333ns     | 2        | 0.222222 | 0.111111ns     |
| Within plots        | 6        | 3.333333 | 0.555556       | 6        | 2.000000 | 0.333333       |
| Total               | 8        | 4.000000 | 0.500000       | 8        | 2.222222 | 0.2777775      |

| Source of variation | Parent 1 | Parent 2 | Generation F₃ |
|---------------------|----------|----------|----------------|
|                      | FD       | SS       | MS             |
| Between plots       | 55       | 252.078993 | 4.583254** |
| Within plots        | 98       | 138.33617 | 1.411594      |
| Total               | 153      | 390.414300 | 2.551727     |

FD: freedom degrees, SS: sum of squares, MS: medium square, ns: no significant at 5% of error probability. * and **: significant at 5 and 1% of error probability by F test.
For broad-sense heritability, the estimates for the two variables studied were high, indicating that most of the variations obtained in these populations are of genetic origin. According to Stansfield (1974), heritability values greater than 0.5 are considered high, and values between 0.2 and 0.5 and less than 0.2 are considered medium and low, respectively.

Narrow-sense heritability is more useful because it quantifies the relative importance of the additive proportion of the genetic variance, which can be passed on to the next generation. The important thing in the evaluation of heritability is to know how much of the selection differential is expected to be realized in the next generation (Falconer & Mackay, 1996).

For total narrow-sense heritability, estimates were 0.494731 for white belly and 0.375862 for amylose. When narrow-sense heritability is high, selection in the early generations of self-fertilization is effective. On the other hand, with a low value, selection should only be practiced in the more advanced breeding generations, since the increase in homozygosity, a consequence of self-fertilization, increases narrow-sense heritability, especially within families (Robinson, 1963; Falconer & Mackay, 1996).

For narrow-sense heritability between families, estimates were 0.700026 and 0.594910 for white belly and amylose, respectively. For narrow-sense heritability within families, these estimates were 0.179276 and 0.117119, respectively. The estimates indicated that the selection between families would be more effective in the F2 generation, showing higher values. For amylose, families with the highest averages would be selected, excluding the selection within families and considering only the selection between families. Selection of families for the trait white belly would be based on the lowest averages of the families.

The traits white belly and amylose content are important parameters that serve as guidelines for plant breeding, as they are agronomically important, being linked to consumer acceptability both by the visual aspect of the polished grain (white belly rate) or by the cooking aspect (amylose content). For rice commercialization, it is necessary to consider specific requirements of the various segments involved in the production and commercial processes of this cereal. These processes highlight the physical quality of the grain, expressed by processing yield, grain yield, size, and appearance (Mingotte et al., 2012). Another important physical property related to the quality of rice grains is translucency (Edwards et al., 2017). In many grains, translucency is interrupted by opaque areas in the endosperm, called white belly spots, plastered grains, or plastered areas (Smiderle & Dias, 2008).

Genetic studies show that amylose content is controlled by a major gene, but it is also influenced by the effect of gene dosage and by minor modifiers (Kumar & Khush, 1987). Furthermore, the environment can influence amylose content, mainly due to the high temperature during grain ripening (Hakata et al., 2012).

For amylose content, progenies with averages higher than that of the highest parent (29.44%) will be selected. The selection pressure used between and within families to select families with an average higher than that of the highest parent was 40%.

From the prediction of genetic gains from selection for amylose content, 22 F2 families were selected, whose averages were higher than that of the highest parent (29.44%). The average of these 22 selected families was 29.57%. The average of the 56 families evaluated was 28.33% (Table 4). Streck et al. (2018a) evaluated genetic progress from 1972 to 2016 for quality traits and observed significant genetic gains: 0.20% per year, for percentage of whole grains after breeding; -1.38% per year, for percentage of plastered grains and white belly; -0.77% per year, for total plastered area; -0.08% per year, for total whiteness; and -0.82% per year, for nonvitreous whiteness of grains.

Table 3 - Estimates of variance components and genetic parameters for white center characteristics and amylose content in F3 families of irrigated rice.

| Parameters                                      | White center          | Amylose content     |
|------------------------------------------------|-----------------------|---------------------|
| Phenotypic variance between                     | 0.011397              | 1.667390            |
| Phenotypic variance within                      | 0.007417              | 1.411594            |
| Environmental variance between                  | 0.000901              | 0.074074            |
| Environmental variance within                   | 0.001650              | 0.444444            |
| Genetic variance between                        | 0.011307              | 1.593316            |
| Genetic variance within                         | 0.005767              | 0.967149            |
| Broad-sense heritability between                | 0.992039              | 0.955575            |
| Broad-sense heritability within                  | 0.777548              | 0.685147            |
| Additive genetic variance                       | 0.005319              | 0.661298            |
| Genetic dominance variance                      | 0.017750              | 3.207298            |
| Narrow-sense heritability between               | 0.700026              | 0.594910            |
| Narrow-sense heritability within                 | 0.179276              | 0.117119            |
| Total narrow-sense heritability                  | 0.494731              | 0.375862            |
Table 4 - Gain estimation by selection aiming to increase the amylose content in the F₄ population of irrigated rice.

| Genetic gain between          |          |
|-------------------------------|----------|
| Selection differential        | 1.246    |
| Narrow-sense heritability     | 0.595    |
| Selection gain (%)            | 0.741    |
| Selection gain (%)            | 2.618    |
| Average of the individuals selected (GG) | 29.57 |
| Average of the highest parent | 29.44    |
| Average of the families       | 28.33    |

The analysis of the results for the prediction of selection gain (Table 4) shows positive gains in amylose content for the F₄ generation of irrigated rice. Based on the observed results of narrow-sense heritability, selection in F₃ should be carried out between families, where the estimated gains from selection for F₂ would be 0.741 or 2.618% for amylose. Selection gains are not high for the trait amylose; however, the average of the parents used is high, meeting the standards of the rice market. In this sense, the expected gains in families overcome the values of parents, and even of the highest parent, who already has high amylose content. Although the expected genetic gains from selection are not so high, there was genetic gain for breeding purposes in obtaining families with higher amylose content.

Conclusions

Gelatinization temperature is low (63 to 68 °C) for all evaluated families.

The F₃ families show genetic variation for the traits white belly and amylose, which allows genetic gains from selection via genetic breeding.

Selection for white belly and amylose can be prioritized among F₃ families. Twenty-two (22) families have a higher average amylose content than the highest parent.

The estimated genetic gain for amylose content was 2.618% in families derived from the hybrid BRSCIRAD 302.

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