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Original Article

Reserve proteins in grains of traditional and improved rice varieties in function of nitrogen availability

Mônica Gouvêa Malheiros, André Marques dos Santos*,
Sonia Regina de Souza, Manlio Silvestre Fernandes

Universidade Federal Rural do Rio de Janeiro, RJ, Brasil.
*Autor correspondente, e-mail: andrmarques@yahoo.com.br

Abstract

This article reports of two rice varieties – Piauí (traditional from the Brazilian state of Maranhão) and IAC-47 (improved by a research institution) – regarding grain production and protein reserves under low and high nitrogen availability. At the end of the growing cycle, the panicles were harvested and separated into filled and unfilled grains and rachillas. For the grains, the total weight per pot, 1000 grain weight, number of filled and unfilled grains per pot, dimensions (length, width and thickness) and concentration of total N, crude protein, protein fractions and glutelin/crude protein ratio were determined. Although the level of crude protein and its fractions did not differ between the varieties, under low N availability, the traditional variety was more productive. Therefore, the results indicate greater efficiency in the use of N by the Piauí variety under low availability of this nutrient in the soil.

Keywords: crude protein, glutelin, nitrate, nitrogen use efficiency, protein fractions

Proteínas de reserva em grãos de variedades tradicional e melhorada de arroz em função da disponibilidade de nitrogênio

Resumo

Duas variedades de arroz cultivadas no Brasil, Piauí (variedade tradicional cultivada no estado do Maranhão) e IAC-47 (variedade melhorada por instituições de pesquisa) foram avaliadas quanto à produção de grãos e acúmulo de proteínas de reserva sob baixa e alta disponibilidade de nitrogênio. Ao final do ciclo da cultura, as panículas foram coletadas e separadas em grãos cheios, grãos chochos e raqui. Nos grãos foram determinados o peso total por vaso, o peso de 1000 grãos, número de grãos cheios e chochos por vaso, dimensões (comprimento, largura e espessura) e o conteúdo de N-total, proteína bruta e suas frações protéicas e a relação glutelina/proteína bruta. Embora o teor de proteína bruta e suas frações protéicas não diferiram entre as variedades, sob a menor disponibilidade de N a variedade tradicional apresentou maior produtividade. Sendo assim, estes resultados indicam maior eficiência no uso de N pela variedade Piauí sob condições de menor disponibilidade deste nutriente no solo.

Palavras-chave: proteína bruta, glutelina, nitrato, eficiência no uso de nitrogênio, frações proteicas

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Introduction

Rice serves as a staple food for over half the world's population (Wander, 2006). According to the FAO (2010), rice accounts for around 27% of the energy and 20% of the proteins consumed in developing countries. Since many of these countries have high rates of poverty and undernourishment, rice can be considered an important ally in the fight against hunger.

In Brazil, the area cultivated with rice in the 2011-12 harvest was 2.454 million hectares, with national production of 11.600 million tonnes (metric tons) and average yield of around 4,779 kg ha^{-1} (CONAB, 2012). Rice is of huge importance in the Brazilian diet, especially that of low-income people, by supplying calories and proteins of high nutritional quality at low cost.

However, one of the main limitations of this crop is the low level of total protein in the grains, around 7% (Juliano et al., 1971; Nalivko et al., 1975). Although this protein content is lower than in other cereals, such as corn and wheat, rice has a relatively higher content of glutelin, the highest quality protein fraction. The protein of cereal grains is composed of four fractions: albumin, globulin, prolamin and glutelin. The main protein reserve of rice consists of glutelin, which contains higher levels of the amino acids essential to the human organism, while in other cereals the predominant fraction is prolamin, which has lower levels of these amino acids.

The nutritional quality of rice grains is determined mainly by the crude protein (CP) content (Deckard et al., 1984). Araújo et al. (2006) and Souza et al. (1993) observed that an increase in the CP level is accompanied by an increase in the glutelin fraction, i.e., increased total protein content is accompanied by higher nutritional quality of the grains. This does not occur with other cereals, like *Zea mays* where an increase in the crude protein content of the grains is accompanied by a higher proline fraction and consequently reduced nutritional value.

Nitrogen (N) is an essential element to increase the level of crude protein in rice grains. When low nitrogen availability in the soil limits the grain production of a rice cultivar, the crude protein level increases at the cost of a reduction in the grain yield. Therefore, to produce rice with high CP content without reducing productivity, it is necessary to increase the supply of N, meaning higher production costs. In turn, to enhance rice productivity and nutritional quality at a low cost, it is necessary to increase the nitrogen use efficiency (NUE) of this cereal (Ferraz Junior, 1997). This combination of high grain yield and crude protein levels can be achieved with traditional varieties that are efficient users of nitrogen (Sherrard et al., 1984).

Studies have been performed on traditional rice varieties in the state of Maranhão that apparently have undergone an adaptive process, making them more efficient in utilizing the N accumulated in the initial development stage and thus less dependent on external supplementation of this nutrient during the reproductive phase (Souza et al., 1998; Santos et al., 2003; Rodrigues et al., 2004; Santos et al., 2007). These varieties also have a higher NUE than improved varieties developed by research institutions to respond to nitrogen fertilization. One adaptive process of plants to environments where the availability of this nutrient is low, occurring in seasonal "flush" is rapid absorption and accumulation of nitrate (NO_3^-) in the initial development stages for use during the later stages of plant growth (Souza et al., 1998; Santos et al., 2003; Rodrigues et al., 2004; Santos et al., 2007).

Under conditions of limited nutrients and water, these plants can develop adaptation mechanisms making them more efficient in using the available resources, among them nitrogen. This adaptive response is a way for the plant to obtain sufficient N for development and production of seeds with high levels of crude protein. For humans, this adaptation results in lower production costs, higher protein quality and reduced environmental pollution.

The aim of this work was to determine the concentrations of grain crude protein and the corresponding protein fractions in a traditional rice variety versus an improved one and to associate the protein levels to productivity by correlation to the nitrogen use efficiency.

Material and Methods

The experiment was conducted in

a greenhouse of the Soils Department of the Agronomy Institute of Rio de Janeiro Federal Rural University (UFRRJ), located in the municipality of Seropédica, Rio de Janeiro, Brazil (22°45' South Latitude and 43°41' West Longitude), between August 2006 and February 2007.

To study the protein levels in the rice grains (*Oryza sativa* L.) submitted to different nitrogen doses, two varieties were used, which were distinct regarding the selection process. Piauí is a traditional variety from Maranhão State, mainly grown by smallholders, and is apparently efficient in the use of nitrogen, while the IAC-47 cultivar is an improved variety developed by the Campinas Agronomy Institute to respond to nitrogen fertilization.

The seeds were disinfected with 2% sodium hypochlorite for 20 minutes in an stirrer, washed thoroughly with distilled water and then placed to germinate on gauze in pots containing distilled water, kept in a growth chamber. Five days after germination (DAG), the water in the pots was replaced by a Hoagland solution at one-fourth the ionic strength (IS) (Fernandes, 1983; Fernandes, 1984) and the pots were transferred to the greenhouse. Ten DAG, groups of five plantlets were transferred to pots containing a substrate consisting of Chernosol from the upper 20-cm layer, collected in the Vila Kennedy district of the city of Rio de Janeiro, previously screened through an 8-mm mesh sieve. The soil had the following characteristics: pH 6.0; C 16.2 g kg^{-1}, P 23.2450 and K 3.2226 ($mmol_c$ dm^{-3}); Na 1.35, Ca 63, Mg 41, K 3.2, H+Al 26, Al 0.0, S 108.6 and T 134.6 ($mmol_c$ dm^{-3}); V 81, m 0 and n 1 (%). This substrate was chosen for having high fertility. The plants were culled one month after transplantation, leaving two plants per pot.

Twelve pots were used, arranged randomly on the greenhouse floor. Every week the pots were rearranged at random to reduce the microclimatic effects and achieve greater homogeneity among the treatments. Fifteen days after transplantation (DAT), 150 kg $N\text{-}NO_3^-$ ha^{-1} (0.63g N pot^{-1}) was applied in the form of potassium nitrate (KNO_3) to half the plants of each variety (traditional and improved), to obtain the following treatments: (1) Piauí without N fertilization; (2) Piauí with 150 kg $N\text{-}NO_3^-$ ha^{-1}; (3) IAC-47 without N fertilization; and (4) IAC-47 with 150 kg $N\text{-}NO_3^-$ ha^{-1}. The experimental design was fully randomized with a 2x2 factorial arrangement (composed of the two rice varieties [Piauí - traditional and IAC-47 - improved] and two NO_3^- doses [0 and 150 kg $N\text{-}NO_3^-$ ha^{-1}]), with three repetitions.

Each day the maximum and minimum temperature, relative air humidity and light intensity were measured with a thermometer, thermohygrometer and radiometer, respectively. The readings were taken at 09h00 and 15h00.

At the end of the cycle the panicles were harvested and the grains with hulls were separated from the rachillas and sorted into filled and unfilled grains.

The 1000 grain weight and number of filled and unfilled grains per pot were determined. Then, samples of 30 filled grains from each treatment were measured (length, width and thickness) with a Digimess digital pachymeter to determine the class and shape of the grains according to Fonseca et al. (1982) and Fonseca et al. (1984).

The grains were hulled manually and ground with a mortar and pestle until fine enough to pass through a 60 mesh sieve. The flour obtained was used in the subsequent analyses of total N, crude protein and protein fractions. The unfilled grains, rachillas and hulls of the filled grains (paleae and lemmas) were also ground into flour to measure the total N.

The total N content was determined by the steam stripping method described by Tedesco et al. (1995). The crude protein content of the flour made from the hulled grains was calculated by the value of total N multiplied by the factor 5.95. This factor is based on the nitrogen content (16.8%) of glutelin, the main protein of rice (Juliano et al., 1985). The sequential extraction of the proteins was done according to Souza et al. (1993), with modifications for adaptation to 2-mL centrifuge tubes.

Samples weighing 120 g of the rice flour were placed in 2-mL microtubes and were initially submitted to saline extraction, with 1.5 mL of a solution containing 2.9% sodium chloride and 0.002% sodium-EDTA, followed by orbital agitation for 30 minutes at room temperature and centrifuging at 12,000 g for 10 minutes at 10 °C. The supernatant was separated and the process was repeated twice more. Then the content of albumin + globulin was determined in the final supernatant by the method of Bradford (1976), utilizing Commassie brilliant blue G-250 and albumin from bovine serum as a standard. The next step was alcohol extraction, by adding 1.5 mL of an alcohol solution (50% isopropanol, 41 mM Tris, 40 mM boric acid, 0.6% mercaptoetanol) to the residue from the saline extraction. Finally, alkaline extraction was performed by adding 1.5 mL of an alkaline solution (0.48% boric acid + 0.4% sodium hydroxide) to the residue from the alcohol extraction. The extraction steps were repeated and the protein levels determined until all the glutelin had been extracted, as described previously, but in this case with agitation and resting times at room temperature of 60 minutes each.

The data were analyzed statistically using the Sigmaplot 8.0 program and the means were compared by the F-test at 5% significance or by the standard error.

Results and Discussion

The Piauí and IAC-47 varieties presented different cycles during its development. The IAC-47 variety completed its cycle in 110 days while the Piauí variety did so in 150 days. The emergence of panicles from the IAC-47 variety occurred in a period with maximum daytime temperatures of around 37 °C and nighttime ones of 20 °C, with more intense luminosity at noon and lower relative humidity during the daytime. These maximum daytime and nighttime temperatures were higher for the IAC-47 variety, at 45 °C and 24 °C, respectively, with luminosity high in the morning and decreasing substantially in the afternoon, and higher humidity of around 93% (Table 1).

Table 2 presents the morphological data on the grains of the two varieties under two doses of nitrogen as nitrate. The grains of the Piauí variety were classified as medium and semi-rounded based on their length, thickness and length/width ratio. In contrast, the grains of the IAC-47 variety were classified as long because of their elongated shape. These morphological features were not affected by the nitrogen fertilization for either variety (Table 2).

Table 1. Temperature (mean maximum and minimum), light intensity and relative humidity measured at 09h00, 12h00 and 15h00h during the cultivate of the rice varieties inside the greenhouse.

Procedures and Harvesting	Temperature					Light Intensity			Relative Humidity		
	---------------------°C---------------------					-µmol quanta $m^{-2} s^{-1}$-			-----------%---------		
	Max.	Min.	9 AM	Noon	3 PM	9 AM	Noon	3 PM	9 AM	Noon	3 PM
Transplantation	35	21	34	35	35	680	1200	490	42	44	45
Fertilization	39	21	35	38	38	545	1300	470	41	43	45
Attack by stem bugs	35	21	31	32	32	600	1250	455	54	49	55
Culling	39	18	31	35	37	850	1350	700	47	41	43
Emergence of IAC47 panicles (1PI)	37	20	35	37	32	950	1650	700	41	41	44
Emergence of Piauí panicles (1PP)	45	24	43	41	35	1800	1700	75	83	94	93

Table 2. Thickness, length, width, length/width ratio and weight per thousand grains of the rice varieties (Piauí and IAC-47) with or without N fertilization.

RICE VARIETY	FERTILIZATION (kg $N\text{-}NO_3^-.ha^{-1}$)	TH	L	W	L/W	GW	KWK
PIAUÍ	0	2.00	5.67	3.00	1.89 Ab*	9.95 Aa	28.87 Ab
	150	1.91	5.83	2.93	2.00 Ab	2.60 Bb	28.62 Ab
IAC-47	0	1.99	7.11	2.74	2.60 Aa	7.69 Bb	34.89 Aa
	150	2.07	6.90	2.68	2.57 Aa	16.53 Aa	33.96 Aa

TH = thickness (mm); L = length (mm); W = width (mm); L/W = length/width ratio; GW= grain weight (g).pot^{-1}; GWK = weight of a thousand grains (g). * Means followed by the same letter (capital between nitrate doses and small between varieties) do not differ according to the F-test at 5% significance.

The traditional variety produced heavier grain weight per pot than did the improved variety in the absence of nitrogen fertilization. However, when the plants received additional nitrogen, the weight of the IAC-47 variety doubled due to the increase in the number of filled grains, while the weight of the Piauí variety declined because of the increased production of unfilled grains (Tables 2 and 3). This higher production of unfilled grains can be attributed to the high maximum daytime temperatures (45 °C), which caused sterility of the grains, since according to Takada (1987), the ideal temperature range for growing rice is 19 to 33 °C. On the other hand, there was no effect of the fertilization on the average weight of 1000 grains of both varieties, although this weight was higher for the IAC-47 variety (Table 2).

In the treatments with nitrogen fertilization, the Piauí variety produced more total grains and filled grains than the IAC-47 variety (Table 3). When the plants were fertilized, the total grain production of the traditional variety declined, with very low output of filled grains, while the total grain production of the improved variety tripled and the number of filled grains doubled (Table 3).

Table 3. Production of grains (total and full) per pot of the rice varieties (Piauí and IAC-47) with or without N fertilization.

RICE VARIETY	TREATMENT (kg N-NO_3^- ha^{-1})	Grains pot^{-1}	Full grains pot^{-1}
PIAUÍ	0	483 Aa	296 Aa
	150	331 Bb	27 Bb
IAC-47	0	238 Bb	205 Bb
	150	681 Aa	419 Aa

* Means followed by the same letter (capital between nitrate doses and small between varieties) do not differ according to the F-test at 5% significance.

The total nitrogen levels of the filled grains, unfilled grains, hulls and rachillas (Figure 1) were similar between the two varieties in the treatments without fertilization. In the treatments that received fertilization, the IAC-47 plants produced higher N content only in the unfilled grains, while in the Piauí variety there was an increase of total N both in the filled and unfilled grains, in both cases higher than in the IAC-47 grains.

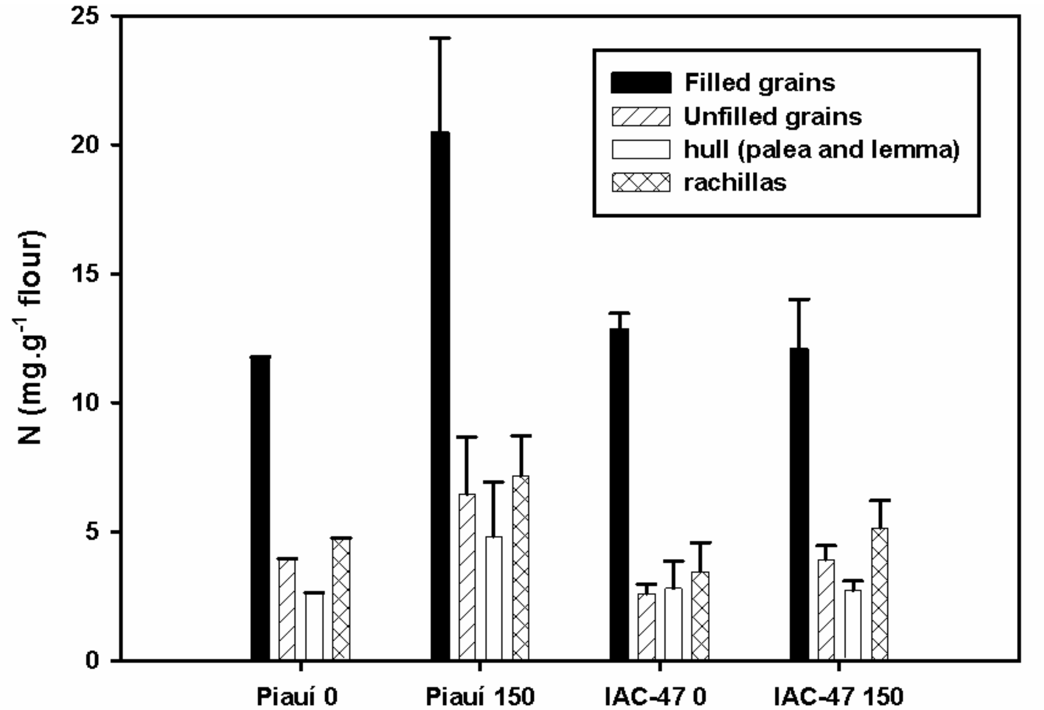

Figure 1. Total N (mg g flour $^{-1}$) of the filled grains, unfilled grains, hull (palea and lemma) of the filled grains and rachillas of two rice varieties (Piauí and IAC-47) with or without 150 kg N-NO_3^- ha^{-1}. Each bar represents the mean value of three repetitions (± standard error).

To extract all four protein fractions (albumin, globulin, prolamin and glutelin) completely, four saline extraction processes were necessary, i.e., three repetitions to extract the albumin + globulin fractions, along with three alcohol extractions to obtain the prolamin fraction and seven alkaline extractions to obtain the glutelin fraction, as shown in Figure 2.

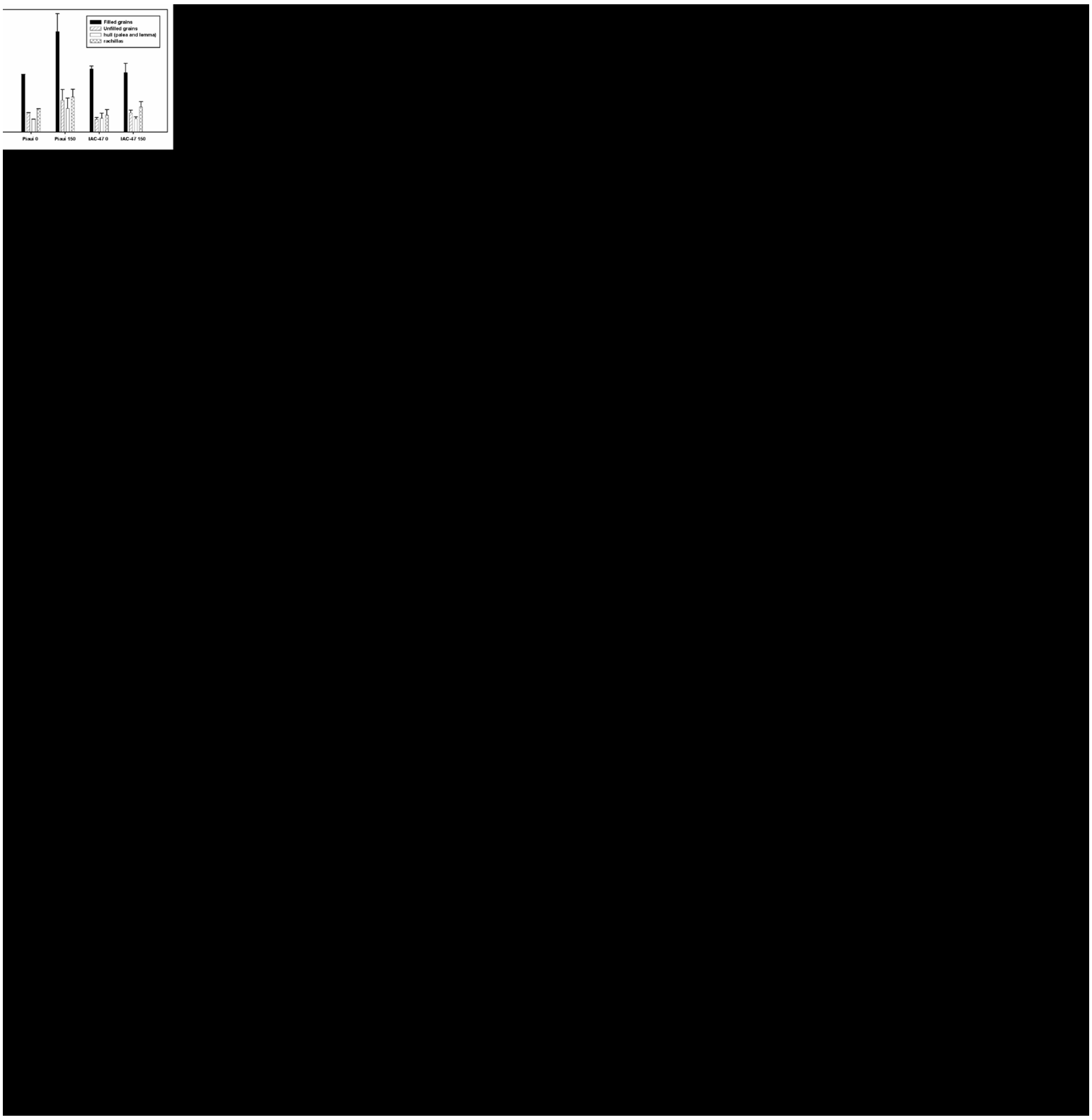

Figure 2. Concentrations of albumin+globulin, prolamin and glutelin according to the steps of saline, alcohol and alkaline extractions, respectively, from the grains flour of two rice varieties with and without nitrogen fertilization. 1st, 2nd, 3rd, 4th, 5th, 6th and 7th extraction steps. Each bar represents the mean value of three repetitions (± standard error).

In both varieties, the grains contained higher levels of glutelin than prolamin, as expected for rice, confirming the higher nutritional quality of rice in comparison with other cereals such as corn, in which the main protein is prolamin, with lower nutritional quality (Figure 3). This same finding was reported in rice grains by Lásztity, (1986), Souza (1995), Ferraz Junior et al. (2001) and Araújo et al. (2003).

In the treatments without nitrate, there was no difference in the levels of crude protein and its fractions between the two varieties (Figure 3), but the Piauí variety produced more grains (296 filled grains per pot) than the IAC-47 variety (205 filled grains per pot). Besides this, the total grain production (filled and unfilled grains) produced by the Piauí variety was double that of the IAC-47 plants (483 versus 238 grains per pot) (Table 3). These results indicate that the traditional variety, in soils with lower available N, is more efficient in using this nutrient because these plants produce more grains with lower soil nitrogen availability.

Ferraz Junior et al. (2001) observed that when 10 rice varieties from Maranhão (among them Piauí) classified as producing grains with high protein content were cultivated in Seropédica, RJ, there was lower crude protein content and higher grain production in the controls without N. The authors attributed this result to the greater luminosity and higher temperatures in Rio de Janeiro than in Maranhão during the experimental period. When fertilized with nitrogen by leaf spraying, these varieties presented increased levels of crude protein and reduced grain production.

In the treatments that received 150 kg N-NO_3^- ha^{-1}, the filled grain yield of the IAC-47 plants doubled (419 grains per pot) and the total grain yield tripled (681 grains per pot) (Table 3), while the protein levels remained the same (Figure 3). This increase in productivity was obtained because IAC-47 is an improved variety, bred to be responsive to fertilization. This did not occur with the Piauí variety, which produced 331 total grains per pot but only 27 filled grains per pot (Table 3). Thus, this treatment resulted in a high level of crude protein and glutelin, since there were few sinks. Patrick et al. (1974) observed that the application of nitrogen before anthesis caused an increase in the protein content but reduced the grain production. The production of unfilled grains in the present study can be explained by the high temperatures (above 35 °C) during the panicle emergence period. It can also be associated with the high luminosity in the period, or even with the presence of rice stalk b'ugs observed during the experiment, mainly on the plants receiving the nitrogen because of the greater number of sprouts.

Since the cycles between the varieties were different, with 150 days for the Piauí and 110 days for the IAC-47 variety, the former variety was subjected to more severe stresses than the latter, hence producing more unfilled grains and having lower overall productivity. The high temperatures, both daytime and nighttime, during the panicle emergence period and presence of rice stalk b'ugs of the Piauí plants appears to be the main factor responsible for the lower grain yield of this variety. According to Juliano (1985), the crude protein levels in rice grains can vary by up to 7 percentage points due to changes in environmental conditions.

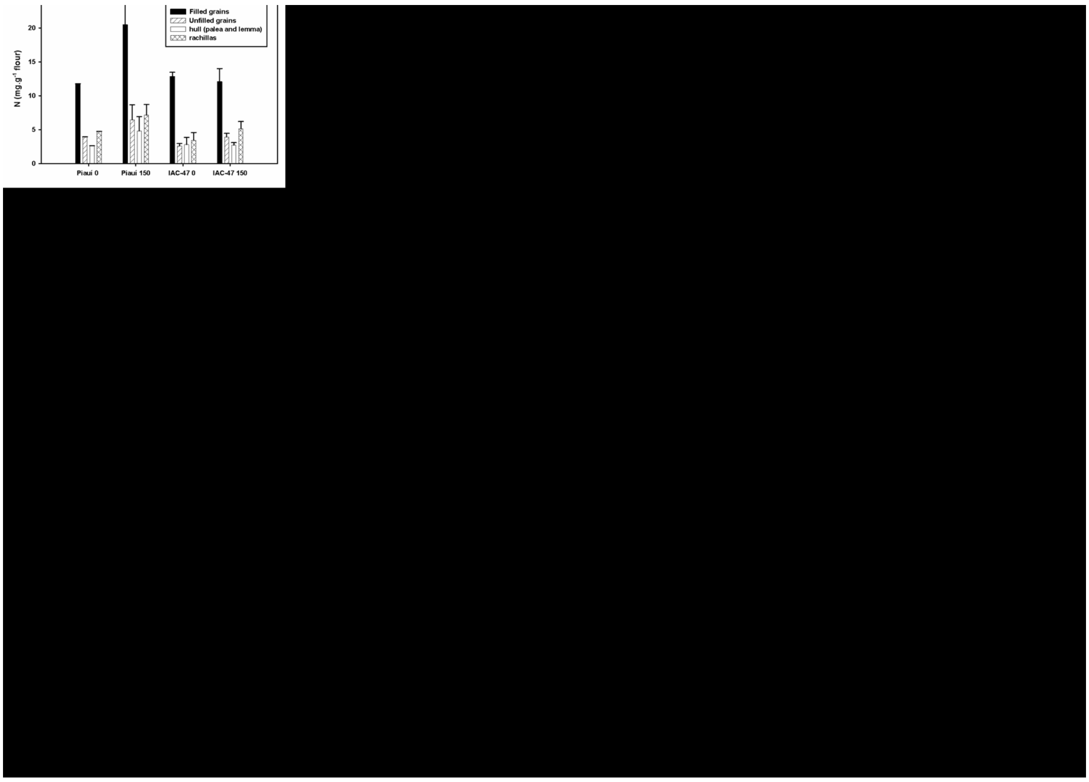

Figure 3. Crude protein and protein fractions (glutelin, albumin+globulin and prolamin) (mg of protein g $flower^{-1}$) of the grains of two rice varieties (Piauí and IAC-47) with and without fertilization with 150 kg N-NO_3^- ha^{-1}. Each bar represents the mean of three repetitions (± standard error).

There was the positive correlation between the concentrations of crude protein and glutelin (r = 0,79), indicating that an increase in crude protein in rice grains is accompanied by a rise in the glutelin fraction, which has greater nutritive value. The same results were reported by Araújo et al. (2006), Ferraz Junior et al. (2001) and Souza (1990), Souza et al. (1993). This is in contrast to corn, for example, in which an increase in crude protein is accompanied by increased levels of prolamin fraction, leading to reduced nutritional quality, because this fraction has lower levels of essential amino acids.

Conclusions

In conclusion, the Piauí variety was more efficient in using the nitrogen available in the soil to form and fill the grains than was the IAC-47 variety. Although the traditional variety produced the same quantity of crude protein and protein fractions as the improved variety, its productivity was higher under conditions of lower nitrogen availability. The results of the crude protein and reserve proteins in the grains of each rice variety show the close relationship between glutelin and crude protein, i.e., an increase in the glutelin content is related to an increase in the grain quality.

These results indicate the traditional variety is more efficient in using nitrogen under conditions of low availability of this nutrient in the soil.

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