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# Patterns of physiological parameters and nitrogen partitioning in flag leaf explain differential grain protein content in rice

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ABSTRACT

The grain protein content (GPC) in rice is low, and more efforts with agronomic and molecular approaches were performed to increase them. However, the rice research focusing on the plant physiological behaviour that modulates the phenomenon of grain protein filling is very scarce. This work contains physiological parameters related to photosynthetic activity in the flag leaf in the grain filling period and N partitioning assays of high (Nutriar) and traditional (Camba) GPC cultivars. Results indicated a higher photosynthetic capacity, a better capacity to provide $CO_2$ to the chloroplast and a healthier PSII structure in Camba relative to Nutriar. Chlorophyll fluorescence parameters decreased more steeply over time in the high protein variety, and a strong negative correlation was observed between GPC and PSII structure parameters. N content in the flag leaf at anthesis showed lower values and higher remobilisation during the grain filling period in Nutriar compared to Camba. The results of this work suggested that the inactivation of some PSII structures in higher GPC cultivars is associated with N remobilisation and would contribute to an increase in the free N available to be translocated to the grain.

## 1. Introduction

Rice (Oryza sativa L.) is a staple food for more than half of the world population (Chattopadhyay et al. 2019a) and contributes 60% of protein intake in Asia (Shih, 2003). The nutritional importance of rice grain protein is its primary composition based on prolamins and glutelins (Ufaz and Galili, 2008.; Zhang et al., 2008) and amino acid composition comparable to the profile of milk casein and soybean protein (Wang et al., 1999).

Grain protein content (GPC) values range between 3.5 at 18.2% within a large group of rice genotypes with wide genetic diversity, revealing a high GPC variability in this species and significant growing-condition dependence (Chattopadhyay et al. 2019a). However, in practically all commercial rice varieties, GPC is low, ranging between 7 and 9% (Traditional GPC) based globally.

Research in GPC breeding is limited by the negative correlation between yield and protein content in cereal grains (Simmonds, 1995), a phenomenon widely reported in rice (Vasal, 2002). In addition, there is also a negative relation between GPC and cooking quality that further complicates the breeding by GPC (Chattopadhyay et al., 2019b). Significant advances on molecular bases to explain the genetic architecture of GPC in rice were made by genome-wide association analysis, where seven molecular markers were associated with the GPC trait (Zhao, 2011). Also, other authors described a multi-environmental QTL associated with GPC (Chattopadhyay et al., 2019a) and a putative amino acid transporter (Peng, 2014). However, there is little knowledge about the relationship between flag-leaf (FL) physiology and GPC.

An association has been described between stomatal conductance (gs) and the nitrogen content of rice flag leaves (Yoshida and Coronel, 1976; Hubbart et al., 2007). However, an in-depth study was not made

*Abbreviations:* γRC, Fraction of RC chlorophyll in relation to total chlorophyll; ABS, Absorbance; BG, Brown rice; Chl, Chlorophyll; CS, Excited cross-section; CT, Canopy temperature; DAA, Days after anthesis; $\Psi E_0$, Electron transport; FL, Flag leaf; $F_V/F_M$, The maximum quantum yield of PSII photochemistry; GPC, Grain protein content; gs, Stomatal conductance; GY, Grain yield; Pn, Net photosynthetic rate; RC, Active PSII reaction centre; RC/CS, number of active reaction centres per active cross-section; SL, Stem and other leaves.

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on rice FL physiology using cultivars with contrasting GPC during grain-filling to analyse this phenomenon. In line with this, the leaf N content is closely related to the photosynthetic apparatus's physiological functionalities and structure. This phenomenon is because the N is one of the main constituents of the chloroplast in every plant species included rice (Laza et al., 1993), and plants devote more than 70% of their available N to maintaining chloroplast function (Makino and Osmond, 1991). Therefore, fast and non-invasive techniques could help investigate the relationship between GPC and FL physiological parameters at the field level, such as gas exchange analysis, thermographic images, and chlorophyll (Chl) fluorescence.

It has been demonstrated that leaf temperature varies with transpiration from the leaves, and these changes are correlated with gs that provide information about the foliar capacity to supply $CO_2$ to the chloroplast (Jones, 1999; Takai et al., 2010). On the other hand, Chl fluorescence is used to get information about PSII activity, and it allows a detailed description of the state of structures and subprocesses related to the PSII complex (Strasser et al., 2000).

In this work were used morphological, biochemical, and non-invasive techniques were to study various parameters associated with the FL physiology, N percentage (%N) and GPC in plants under field conditions during the grain filling stage, and the hypothesis was that the FL physiological behaviour during the grain filling could explain the contrasting GPC in rice cultivars.

With breeding research over a decade at the National University of La Plata (UNLP, Argentina), we developed the commercial rice cultivar Nutriar FCAyF (Nutriar), considered a high GPC cultivar that is showing 20–30% more protein than traditional GPC rice cultivars, reaching up 11–13% of GPC (Pinciroli et al., 2009; Pinciroli et al., 2019). This cultivar and traditional GPC cultivar Camba-INTA (Camba, García de Salamone et al., 2012; Martínez et al., 2014) were cultivated in two growing seasons where biochemical and physiological parameters were measured. Both cultivars were also grown in a third growing season to perform an N partitioning assay.

## 2. Materials and methods

### 2.1. Plant material, experimental design, and crop management

The experimental cultures were performed at the INTECH institute (35°37′50.47″ S - 57°59′40.41″ O) in three 2.8 $m^2$ × 0.4 m plastic pools filled with soil (pH in water 6.4, organic carbon 2.82% with Walkley and Black method, N 0.37% with the micro-Kjeldahl method and phosphorus (P) 20 ppm with Bray Kurtz Nº1 method, during growing seasons 2016/2017, 2017/2018 and 2019/2020. The experimental design consisted of a randomised complete block with three replicates in the three growing seasons. One-month-old seedlings were transplanted into the pools, and each cultivar occupied three rows per block, and each row contained 16 plants, spacing 10 cm between plants and 20 cm between rows. The transplant date was 11/22 for the first year, 11/27 for the second year and 12/11 for the third year of cultivation.

The trial was conducted under flooding with rainwater throughout the crop cycle. No plagues were registered during the experiments, and weeds were manually controlled. The amounts of fertiliser applied as a basal dressing were 6.84 g N $m^{-2}$, 2.7 g P $m^{-2}$ and 8.5 g K $m^{-2}$, equivalent to 68, 27 and 85 kg/ha of N, P and K, respectively.

Anthesis was 72 and 79 days after transplanting in Nutriar and Camba respectively in growing season 2016/2017, whilst in the growing season 2017/2018, anthesis occurs at 81 and 90 days for Nutriar and Camba, respectively.

In the first two years, a study was done to evaluate the FL physiology of contrasting GPC cultivars, while in the third year, an analysis of N partitioning was performed. For the physiology study, all parameters were determined at anthesis, 15 and 30 days after anthesis (DAA) on the FL of the main stem.

At the maturation stage, rows were harvested, and panicles were threshed manually; afterwards, the grain was dried in an oven at 40 °C until 14% humidity measured with a grain humidity meter (Wile 55, Wile, FI).

For the N partitioning study, plants were taken at the end of the tilling stage, at anthesis and in the grain maturation stage and separated into four and five fractions to anthesis and maturation stage, respectively.

### 2.2. Determination of biochemical and morphological parameters on the FL

#### 2.2.1. Determination of total chlorophyll content

The total Chl content was determined at anthesis by a wet chemistry method, according to Inskeep and Bloom (1985), with slight modifications. FL from plants of each row was randomly collected and frozen. Then 40 mg of ground leaves were macerated in 1 ml of 80% acetone for 24 h in the dark at 4 °C with low-speed shaking. Then, the extract was centrifuged for 10 min at 4000 rpm, and the absorbance of a supernatant sample was measured at 647 nm (A647) and 664.5 nm (A664.5) using a UV/visible spectrophotometer (ZL 5000, Zeltec, AR).

#### 2.2.2. Determination of stomatal density

The FL stomata density was determined at anthesis using a double-sided tape technique. An imprint of the abaxial epidermis of the FL was made with attached double-sided tape to a glass microscope slide. A safety razor blade was used to gradually scraped away the tissue above the epidermis. The imprint was observed under a light microscope, and an image was digitised directly from the slide. The stomata were counted in the digital image using the ImageJ software (US NIH, Bethesda, MD, USA).

### 2.3. Determination of physiological parameters in FL

#### 2.3.1. Gas exchange parameters

The gas exchange parameters were determined at the middle portion of the main stem flags leaves of three plants in each cultivar per row. The net photosynthesis rate and stomatal conductance were measured between 10:00 and 13:00 h using a portable gas exchange system (TPS-2 Portable Photosynthesis System, MA, USA), at light saturation (1200 mol $m^{-2}$ $s^{-1}$ illumination led light) when the concentrations of $CO_2$ and $H_2O$ were stabilised inside the chamber. The $CO_2$ level in the atmospheric air was around 390 ppm.

#### 2.3.2. Canopy temperature

Parallel to the gas exchange determinations, the temperature at canopy level was also determined between 10:00 and 14:00 h using a portable infrared thermal imaging camera (FLIR E−30, FLIR Systems, USA; thermal sensitivity <0.1 °C, field of view of 25° × 19°) and the emissivity was set as 0.95. The computed temperature represents the mean of two thermal images recorded for each row per plot. Thermal images were processed by ThermaCam Research Pro software (FLIR Systems, USA).

Air temperature data were recorded every 30 min by a weather station (Cavadevices, AR) located in the experimental site.

#### 2.3.3. Fluorescence emission kinetic

OJIP test was performed by measuring Chl a fluorescence at the middle portion of FL of the main stem between 17:00 and 19:00 h using a portable Chl fluorometer (Pocket PEA, Hansatech Instrument, UK). For this purpose, leaves were covered with leaf clips provided by the manufacturer to adapt them to darkness for 20 min. Then, leaf clips were opened, and samples were exposed during 3 s–3500 μmol photons $m^{-2}$ $s^{-1}$ (637 nm peak wavelength). The raw Chl fluorescence data were processed using PEA plus v1.1 software (Hansatech Instrument Ltd., UK) to obtain OJIP parameters associated with PSII properties according to Strasser et al. (2000) and described in detail in Table 1.

**Table 1**
Summary of OJIP parameters, formulae and their description using data extracted from chlorophyll *a* fluorescence analysis.

| parameters | Description |
|---|---|
| $F_V/F_M = (F_M - F_0/F_M)$ | Efficiency of the primary photochemistry, where $F_0$ (Minimum fluorescence at t = 0) and $F_M$ (Maximum fluorescence) represents the situations when all RC of PSII is open and closed, respectively. |
| $\psi E_0 = 1 - V_J$ | Efficiency of the electron transport further than $Q_A$, where $V_J$ represents the relative variable fluorescence at 2 ms ($V_J = F_{2ms} - F_0/F_V$). |
| $RC/CS_0 = F_V/F_M$. ABS/CS. Ss | Amount of reaction centres (RC) per excited area (CS), where ABS/CS represents the photon flux absorbed (ABS) by the pigments (antenna Chl) per CS, and Ss the normalised area of the first fluorescence emission kinetic section. |
| γRC= (1/ABS/RC)/(1+ (1/ABS/RC)) | Fraction of RC chlorophyll in relation to total chlorophyll, where ABS/RC represents the ABS by the antenna Chl per RC. |

### 2.4. Determination of grain protein content

Data of GY was calculated as the mean of the total grain weight per area in each plot. Then, grain samples of each row were milled and sieved using a Cyclone mill 0.4 mm mesh. GPC was determined by the micro-Kjeldahl method (IVA S.A., AR). Shortly samples (50 mg) of milled brown rice were digested with 1.94 g of a catalytic mixture (10:1 $K_2SO_4$: HgO) and 2 ml of $H_2SO_4$. Then the mixture was put on a digestion furnace at 350 °C in a gas hood until the complete digestion of the samples. The digest was distilled after adding 10 ml of NaOH 40% p/v, and ammonium was collected in an Erlenmeyer flask contained 5 ml of 4% w/v $H_3BO_3$ with a few drops of mixed indicator (methyl red and bromocresol green). Then, a titration was done with 0.02 N HCl until that colour turned green to pale pink. The percentage of N (%N) was calculated using the equation:

%N = ((ml of standard acid – ml of blank) x Normality of acid) x 1.4007/ weight of sample (g).

The factor 5.95 was applied to determine grain protein content in rice samples (Juliano, 1985).

### 2.5. Determination of N partitioning

Nutriar and Camba cultivars grew in the 2019/2020 growing season, and samples were taken at the end of the tilling, anthesis, and the maturation stage. Entire plants were harvested in the tilling stage, while plants at the anthesis and the maturation stage were separated into four and five fractions. The plant fractions consist of a panicle (P) that including rachis and glumes, flag leaf (FL), second leaf after flag leaf (FL-1), stem and other leaves (SL) and dehusked grain or brown rice grain (BG). Each sample was dried at 70 °C until constant weight in an oven, and nitrogen was measured as is described in 2.4 with slight modification for semi-micro Kjeldahl using a distillation equipment Tecator 1002 (Foss AB, SE).

### 2.6. Statistical analysis

Data were subjected to ANOVA and post hoc analyses, DGC tests (Di Rienzo et al. 2002) and Student's t-test using the INFOSTAT statistical software package (InfoStat version 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina (http://www.infostat.com.ar). The linear regression analyses and graphics were performed using the GraphPad scientific graphing software (GraphPad Prism Software v6.0 Inc., CA, USA).

## 3. Results

### 3.1. Biochemical and morphological differences between cultivars at the beginning of the grain filling stage

The Chl content and the stomatal density determinations in FL showed significant differences between cultivars in both parameters at the beginning of the grain filling stage. The Chl content in Nutriar (high GPC cultivar) was 54% lower than that registered in Camba (traditional GPC cultivar) in the first growing season and 20% lower in the second growing season, suggesting differences at the photosynthetic apparatus level (Fig. 1). Also, the stomatal density was approximately 16% lower in Nutriar concerning Camba cultivar (629 vs 728 stomatal. $mm^{-2}$, respectively).

This first approach suggested that these cultivars could also show contrasts in physiological terms as gas exchange parameters associated with the photosynthetic performance or different functional and structural PSII parameters derived from the Chl fluorescence analysis favouring the traditional GPC cultivar.

### 3.2. High GPC cultivar showed lower physiological performance than traditional GPC cultivar

Canopy temperature (CT), Pn, gs, and OJIP parameters, were determined at anthesis and intermediate and late time of the ripening stage (15 and 30 days, respectively) in both growing seasons. Remarkably, the CT, Pn and gs results indicated significant differences between cultivars during the ripening stage in both growing seasons.

Inline, thermal images indicated that the CT values in Nutriar were 0.5–1.1 °C higher than in Camba during the anthesis and all-grain filling period in the two-growing season (Fig. 2). Posterior gs determinations also supported the CT and stomatal density results, with gs showing lower values in Nutriar compared to Camba at anthesis time and during the remaining measurement times in both growth seasons (Fig. 3a, b and 3c). The Pn determination at anthesis, 15 and 30 DAA indicated lower values in Nutriar compared to Camba (Fig. 3d, e and 3f, respectively) in

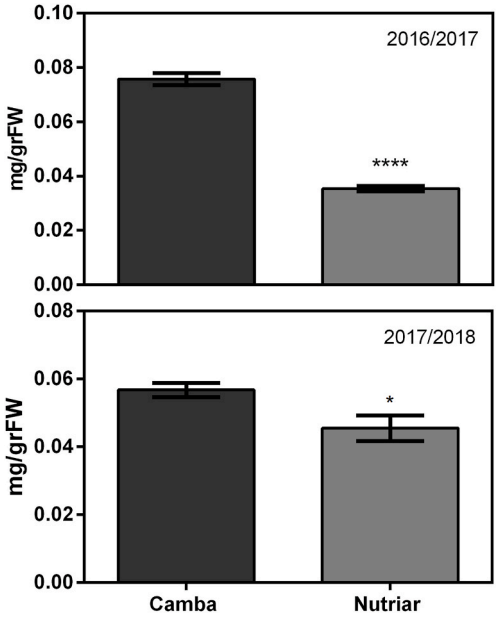


**Fig. 1.** Total Chlorophyll content in flag leaf.
Total chlorophyll content was determined in the FL of Camba (black bars) and Nutriar (greys bars) plants in anthesis. Data were expressed in Chl concentration based on the per-unit fresh weight of FL. Asterisks represent significant differences between treatments (Student t-test; *$p < 0.05$, ****$p < 0.0001$; Data represents mean ± SEM; n = 27).

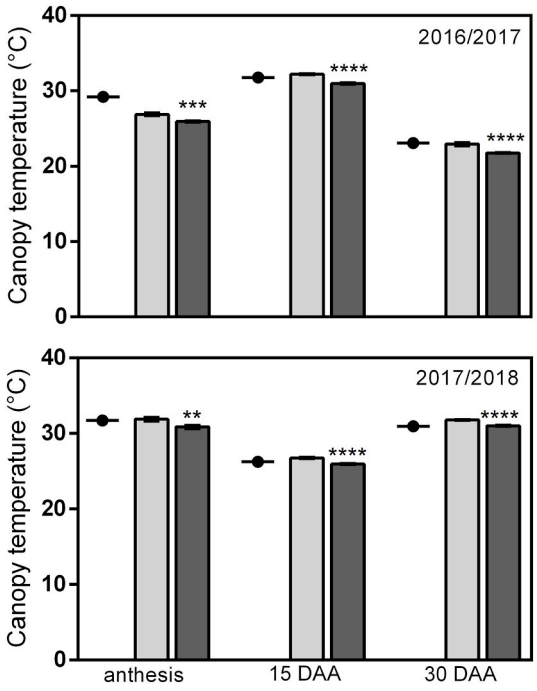


**Fig. 2.** Canopy temperature.
Canopy temperature in Celsius degree of Nutriar (greys bars), Camba (dark bars), and ambient temperature (black dots) were determined at anthesis, 15 DAA, and 30 DAA. (Student t-test; **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant; Data represents mean ±. SEM; n = 9).

both growing seasons. Also, significant positive correlations of Pn and gs were observed during the grain filling stage for both cultivars, except at 30 DAA for the 2017/2018 growing season (Table 2). A significant and strong negative correlation was found between CT and gas exchange parameters Pn and gs, and the correlation coefficients were significant for stages understudy, with some exceptions in both cultivars at 30 DAA (Table 2).

The lower values registered in Nutriar compared to Camba in parameters directly associated with the photosynthetic apparatus, such as the content of Chl and Pn, suggested that the high GPC cultivar could also present contrasts with the traditional GPC cultivar in terms of functional and structural properties of the PSII. Therefore, we performed a parallel determination of PSII activity by Chl a fluorescence analysis using a portable Chl fluorometer. Both cultivars showed similar JIP parameters values at anthesis, and some differences between cultivars in a time-dependent manner were observed at 15 and 30 DDA (Fig. 4). Inline, structural PSII properties as the number of RC density (RC/CS) and the fraction of RC Chl about total Chl (γRC) described a detriment of the PSII structure in Nutriar concerning Camba during the grain-filling period. Typical functional properties determined by the OJIP test as the efficiencies of the primary photochemistry ($F_V/F_M$) and the electron transport ($\Psi E_0$) also did not show significant differences between cultivars during the grain filling period in both growing seasons (Fig. S1).

### 3.3. Negative correlations between GPC with FL photosynthetic parameters suggested an association between GPC with the health of PSII structure

The GPC values were significantly higher in Nutriar than Camba, with an average of 38% and 33% in both growing seasons (Table 3) and parallel, both cultivars presented their higher grain yield values in the first growing season, but there were not grain yield differences between cultivars at this time. However, the grain yield was 30% higher in Camba than in Nutriar in the second growing season.

The GPC data were used to perform different linear correlation analyses between GPC with the other FL physiological parameters (Table 4). In general terms, GPC correlated negatively with physiological parameters, moderately with Pn and moderately to high with JIP parameters. The largest cluster of high correlations was determined, mainly at 30 DAA, where RC/CS and γRC presented the higher correlations. Therefore, the GPC, RC/CS and γRC data were studied by linear regression analysis (Fig. 5). The statistic study indicated that γRC was the physiological parameter primarily associated with GPC because it explained 45% of the GPC variation data (Fig. 5B). Even more, this study allowed us to visualise data clusters corresponding to Nutriar and Camba plants in all cases, suggesting a differential association between GPC with PSII structures in the FL of both cultivars.

### 3.4. N partitioning during the grain filling stage supported the correlations between GPC and physiological parameters

A posterior field assay to determine N partitioning in different plant fractions during tilling, anthesis and the maturation stages was performed in the 2019/2020 growing season. In the tilling stage, neither differentiate between both cultivars was observed with 2.27% and 2.4% N in Camba and Nutriar, respectively, but N content at anthesis was significantly higher in Camba than Nutriar (Fig. S2). These cultivars accumulate N in the different fractions at anthesis, whilst the concentration of N in Camba was higher in FL (9%) and SL (23%), than Nutriar, the higher GPC cultivar showed higher N in P (13%) and FL-1 (17%) compared to Camba (Fig. 6). N content data in FL agree with chlorophyll content data at the anthesis in the previous growing seasons, as is indicated in Fig. 1, but at the maturation stage lowering until 20% compared to Camba. Further, all fragments of Nutriar at the maturation stage showed a mean of 21% lower values in N concentration regarding Camba, and grain showed a higher % N value (32%) in Nutriar than in Camba, according to the previous GPC observed in the earlier growing seasons. (Fig. 6).

On the other hand, a decrease in N content data between anthesis and the maturation stage show intense remobilisation towards grains with drop values of 81% in SL, FL-1, and FL in Nutriar, compared with 79, 69 y 78% in Camba, respectively (Fig. 6).

## 4. Discussion

One of the present work aims to find differences in morphological, biochemical, and physiological parameters determined on the FL because the studies on this analysis in cultivars with contrasting GPC are scarce.

The total Chl content was one of the first determinations that partially shows the plant photosynthetic properties, indicating the light-harvesting capacity (Elfeky et al., 2007). The results of total Chl content and stomatal density determinations in FL at anthesis suggested that the traditional GPC cultivar presented a better photosynthetic condition at the beginning of the grain-filling period compared to Nutriar. Other physiological determinations as Pn, gs and CT also supported this hypothesis at anthesis and during the grain-filling period as indicatives of higher photosynthetic capacity and better ability to provide more $CO_2$ flux to the chloroplast in Camba than in Nutriar. In this regard, several reports indicate that FL photosynthesis contributes 60–100% of the photoassimilates during the grain filling period in rice (Yoshida, 1981; Wada, 1993; Takai et al., 2005). At the same time, Ohsumi et al. (2007) report considerable genotypic differences in Pn and observed differences in gs in rice.

Some authors associate leaf temperature and stomatal density with higher Pn in different plants (Hatfield et al., 1987) and rice (Rebecca et al., 2010; Kondamundi et al., 2016). Furthermore, other reports indicate that Pn in rice is highly determined by gs (Takai et al., 2010; Kanemura et al., 2007). Previous works have demonstrated the relations between CT and gs in rice at tillering stages, but little is known about this

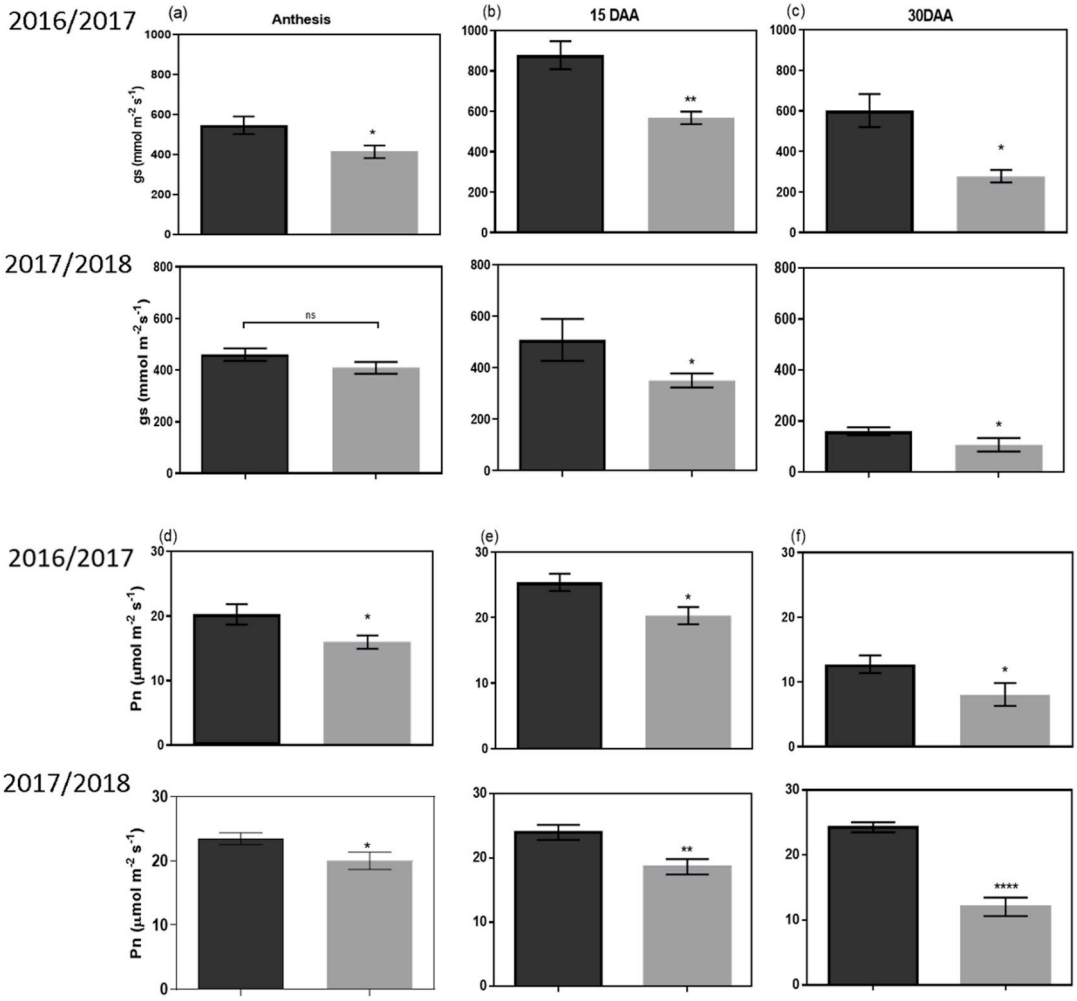


**Fig. 3.** Gas-exchange parameters in flag leaf.
Stomatal conductance (a, b and c) and net photosynthesis rate (d, e, and f) were determined in the FL of Camba (dark bars) and Nutriar (greys bars) plants at anthesis, 15 DAA, and 30 DAA in (A) 2016/17 and (B) 2017/18 growing seasons. Asterisks represent significant differences between treatments (Student t-test; *$p < 0.05$, **$p < 0.01$, ****p < 0.0001, ns = not significant; Data represents mean ±. SEM; n = 27).

**Table 2**
Correlation analyses between gas exchange parameters and canopy temperature. Data represent the Pearson correlation coefficients to each correlation (*p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant; n = 27).

| | gs vs Pn | | | CT vs gs | | | CT vs Pn | | |
|---|---|---|---|---|---|---|---|---|---|
| Season/DAA | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| 2016/2017 | | | | | | | | | |
| Nutriar | 0.78*** | 0.64** | 0.64* | - 0.93*** | - 0.76* | - 0.78ns | - 0.71* | - 0.85** | - 0.8* |
| Camba | 0.58* | 0.6** | 0.67** | - 0.9** | - 0.92** | - 0.78* | - 0.74* | - 0.91*** | - 0.74* |
| 2017/2018 | | | | | | | | | |
| Nutriar | 0.67*** | 0.43* | 0.48ns | - 0.85** | - 0.81** | - 0.36ns | - 0.81* | - 0.81* | - 0.87ns |
| Camba | 0.6** | 0.58** | 0.50ns | - 0.73* | - 0.85* | - 0.76ns | - 0.82** | - 0.7* | - 0.89* |

relationship in rice for grain filling period among cultivars with contrasting GPC. The results confirmed the relationships between CT, gs and Pn during the grain-filling period and showed strong correlations between some of them in the two cultivars studied, suggesting that these parameters also would be related with GPC. This hypothesis was supported by the correlation analyses between gas exchange parameters with GPC, particularly with Pn, and the first indication that the GPC was potentially linked to the photosynthetic state of the FL. This conclusion agrees with the earlier reports that described an association between gs and the N content of rice FL (Yoshida and Coronel, 1976; Hubbart et al., 2007).

Furthermore, the RC density reflects the photochemistry capacity of the photosynthetic apparatus (Gazquez et al., 2018; Bordenave et al., 2019), and in this regard, the evidence obtained in our study showed that RC density values were higher in Camba compared to Nutriar. The result of this work suggested an accelerated detriment of the PSII structures determined by RC/CS and γRC in Nutriar compared to Camba during the grain-filling period. Moreover, GPC correlated negatively with the OJIP parameters RC/CS and γRC, which supported the hypothesis that high GPC cultivar generated a great N source in the FL during the filling period from the inactivation of the PSII structure. Therefore this would allow increasing the N in grain by translocating N

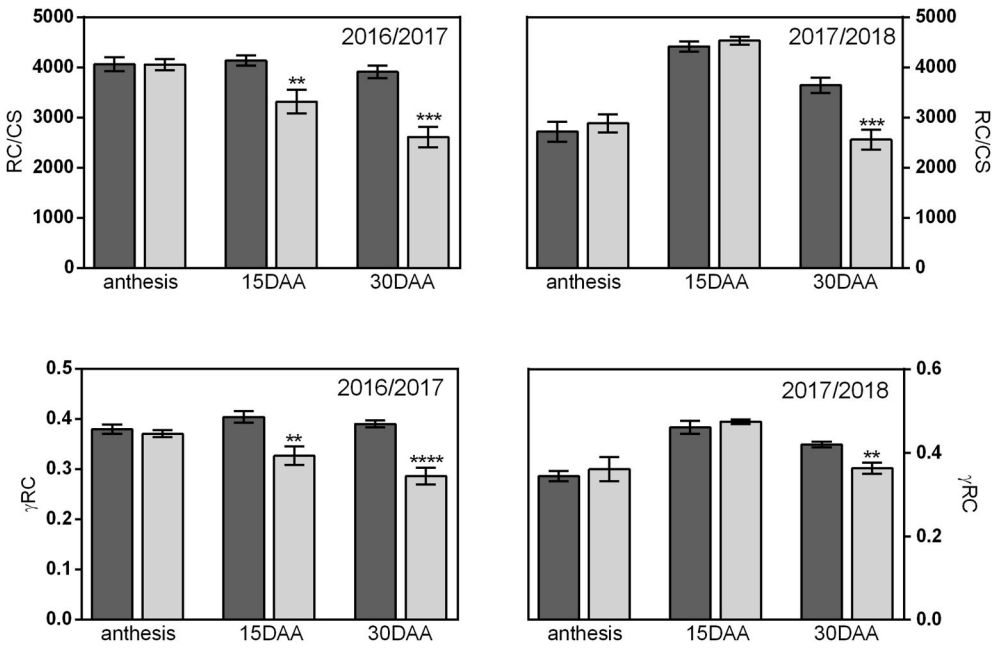


**Fig. 4.** OJIP parameters in flag leaf.
Changes in RC/CS and γRC parameters are derived from the OJIP test. The measurements were performed in the FL of Camba (dark bars), and Nutriar (grey bars) plants at anthesis, 15 DAA, and 30 DAA during the 2016/17 and 2017/18 growing seasons. (Student t-test; **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant; Data represents mean ± SE, n = 27).

**Table 3**
Grain yield (GY) and grain protein content (GPC) among both cultivars in the 2016/17 and 2017/18 growing seasons. Data represents mean ± SEM; n = 3, and different letters significant differences (paired *t*-test *p < 0.05).

| Year/Cultivar | GY (gr $m^{-2}$) | GPC (%) |
|---|---|---|
| Nutriar | 807.1.0 ± 75.6 a | 10.3 ± 0.7 a |
| Camba | 935.2.0 ± 94.2 a | 6.4 ± 0.4 b |
| | | |
| Nutriar | 440.7 ± 17.03 b | 9.7 ± 0.6 a |
| Camba | 630.0 ± 13.08 a | 6.5 ± 0.1 b |

**Table 4**
Correlation analyses between GPC with the different physiological parameters. Data represent the Pearson correlation coefficients to each correlation analyses (*p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant; n = 18).

| | GPC | | |
|---|---|---|---|
| Parameters\DAA | 0 | 15 | 30 |
| Pn | −0.27ns | −0.59** | −0.44* |
| Gs | −0.39* | −0.30ns | −0.29ns |
| RC/CS | 0.11ns | −0.29ns | −0.56*** |
| γRC | 0.01ns | −0.33ns | −0.67*** |

from inactive chloroplast structures, and the %N partitioning data also supported this hypothesis.

In N partitioning assay plant fractions, particularly the FL fraction, suffered extensive translocation from vegetative organs toward the grain during the filling stage, mainly in the higher GPC cultivar where PSII structure functions decline sharply compared with Camba.

These differences became wider at more advanced times, suggesting an increase in the inactivation of chloroplast structures in the high GPC cultivar through the progress of the grain-filling period.

Using N-15 in investigations at grain filling period, Norman et al. (1992) showed that a significant amount of N is required, where the translocation of the vegetative organs can be until 86% of the N in panicle and 58% provided of leaf blades and 28% of leaf sheaths and stem. In the same direction, another author found that a significant fraction of the N requirements of the grain is covered with N from the leaf blades (Mae, 1997).

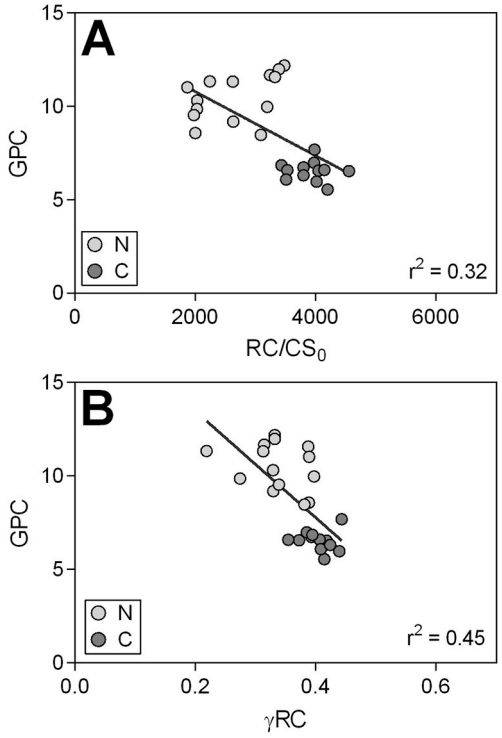


**Fig. 5.** Linear regression between physiological parameters and GPC.
Linear regression analysis between (A) RC/CS and (B) γRC data with GPC data of Camba (C, dark dots) and Nutriar (N, greys dots) plants during the 2016/17 and 2017/18 growing seasons at 30 DAA. The solid line in each graph represents the linear fit model between dependent (Y-axis) and independent (X-axis) variables.

In this work, the drop of N content in plant fractions at the maturation stage related to anthesis showed differential remobilisation in higher GPC cultivar than traditional GPC variety.

Due to the high translocation, lower %N values are found in straw in the higher GPC cultivar than traditional GPC variety at the maturation stage.

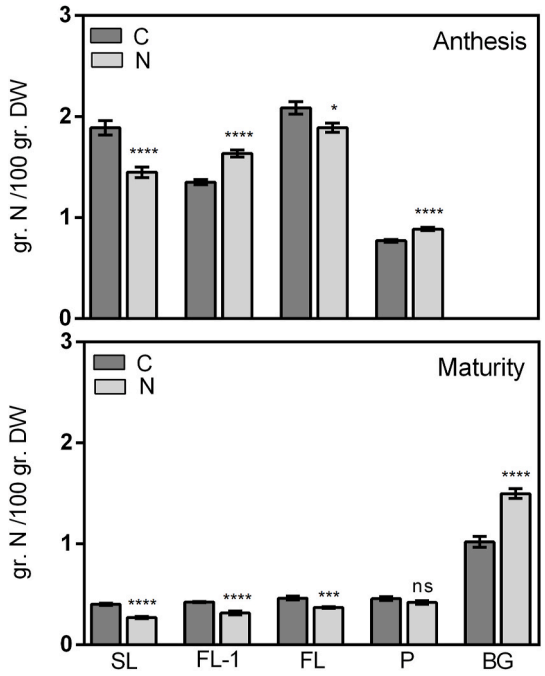


**Fig. 6.** Nitrogen partitioning in anthesis and grain at the maturation stage. Nitrogen concentration (gr. N/100 gr. DW) in plant fraction at anthesis and at the maturation stage in the cultivar Camba (C, dark bars) and Nutriar (N, greys bars) are showed. Flag leaf (FL), second leaf under flag leaf (FL-1), Panicle including rachis and glumes (P), stem and others leave (SL) and dehusked rice grain (BG). Student t-test; *p < 0.05, ***p < 0.001, ****p < 0.0001, ns = not significant; Data represents mean ±. SEM; n = 12).

On the other hand, Huang et al. (2010) found a positive relation between GPC and proteinase activity in the FL of two rice cultivars and their progenies, indicating N remobilising leaves to the grain in higher GPC cultivars. Then, high GPC cultivars would more quickly compromise the structural integrity of PSII than traditional GPC cultivars during grain filling, diminishing the photosynthetic capacity and increasing the N content in the grain at the expense of the non-functional chloroplastic structures recycling. Even more, this hypothesis agrees with different authors that reported the blade of the FL is the primary source of remobilised N that represents 60–90% of the mobilised N in rice panicles (Mae, 1997; Murchie 2002). Also, the Chl and N content was closely related in rice leaves, where 80% of the N is lodged in chloroplasts (Morita, 1980).

Considered, results of the present work suggested that some specific physiological parameters could be determined using quickly and non-destructively techniques as thermographic images and Chl fluorescence as a tool to predict and differentiate rice lines with contrasting GPC in the grain-filling period under field conditions.

In summary, data of this investigation suggest that rice cultivars with high GPC would prioritise incorporating N to the panicle, with great remobilisation of N to the grains that would come from the disassembly of the photosynthetic apparatus, which can be analysed by FL physiology. However, it would be necessary for future research to incorporate more physiological parameters to unravel the physiology of high GPC cultivars in field conditions.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2021.10.034.

## Contributions

MLP, AAR, AAV, RB and SJM: conceptualisation, experimental design and execution.

MLP, AAR and SJM: data analysis and writing.

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