

Non-Structural Carbohydrates Accumulation in Contrasting Rice Genotypes Subjected to High Night Temperatures

Diogo S. Moura¹, Giovani G. Brito², Ângela D. Campos², Ítalo L. Moraes¹, Fabiane G. S. Porto²,
Sheila B. Teixeira¹, Paulo R. R. Fagundes², André Andres², Fábio Schreiber² & Sidnei Deuner¹

¹ Federal University of Pelotas, Campus Capão do Leão, RS, Brazil

² EMBRAPA Temperate Climate, Brazilian Agricultural Research Corporation, Pelotas, RS, Brazil

Correspondence: Giovani G. Brito, EMBRAPA Temperate Climate, Brazilian Agricultural Research Corporation, Pelotas, RS, Brazil. E-mail: giovani.brito@embrapa.br

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Abstract

Non-structural carbohydrates (NSC) accumulation and photosynthesis traits were studied in two rice (*Oryza sativa* L.) genotypes maintained under control (22/30 °C - night/day) and at high night temperatures (HNT) (28/30 °C) conditions from heading to milk stage. Rice cultivars were Nagina22 - N22 and BRS Querência - Quer, which are tolerant and sensitive to high temperatures, respectively. The source-sink flow related attributes were tested to understand the nature of NSC accumulation and translocation. Compared to N22, Quer maintained higher stem starch in glucose on seventh day after heading and at milk stage independently of imposed temperatures. However, the levels of starch in glucose were lower for N22 meanwhile their total sugar concentration (TSC) were higher at control and at HNT at milk stage as compared to Quer. N22 maintained unaltered the spikelet sterility and 1000-grain weight across environments showing a consistent trend with its stem NSC translocation. Both genotypes showed similarity in some gas exchange and chlorophyll fluorescence performance suggesting unaffected photosystem II photochemistry, linear electron flux, and CO₂ assimilation. Beyond indicating that source functioning was not the limiting factor for low TSC and starch in glucose levels found in N22 on seventh day after heading stage. Moreover, our data suggest that the higher translocation capacity shown by N22 can be involved in their lower spikelet sterility and 1000-grain weight stability across the environments. These results indicate that selecting genotypes with higher capacity to stem NSC translocation at HNT could lead to more grain yield stability in future climate scenarios.

Keywords: *Oryza sativa* L., night heat stress, photosynthesis, chlorophyll fluorescence, source-sink flow, yield components

1. Introduction

Rice (*Oryza sativa* L.) is one of the most relevant staple foods for more than half of the world's population (Fan et al., 2016). Thus, rice production must be increased by 70% until 2050 to supply the growing demand for food, take into account the growth population and economic development (Godfray et al., 2010). Additionally, there is a consensus that in the future the climate changes will become a bottleneck for crop yield and its stability (Brito et al., 2010, 2011, 2016; Diola et al., 2011, 2013; Guimarães et al., 2017; Weber et al., 2014). In this way, projected climate changes could reduce crop yields in the future (Fan et al., 2016; Tian et al., 2015). There are differences in the magnitude and uncertainties among the various climate models published and agricultural implications of such extreme climate changes over the twenty-first century. However, historical climatic data show that global temperatures rose by about 0.5 °C between the preindustrial period and 1980-1999. Current climate projections encompass a range of 1.6-6.9 °C for the end of the twenty-first century, relative to the preindustrial period (Sanderson et al., 2010). Although projected impact of climate change on crop yield has been extensively published in the last decade, comparatively, there is still a lack of studies that highlight the role of high nighttime temperatures on rice physiological response and, consequently changes in the rice yield performance.

There are various reports demonstrating the increase in CO₂ concentration in the future, which could lead to a better photosynthesis performance and increases in carbohydrate metabolism enzymatic activity in the source (leaf) in the absence of other bottlenecks. Following this rationale, higher NSC could be accumulated in the sink

(grains) among crop plants. This could greatly increase the number of tillers, panicles, spikelet per panicle and proportion of filled spikelets, also enhancing 1000-grain weight (Shimono et al., 2009; Zhu et al., 2014). On the other hand, there are negative effects from heat stress, especially during the beginning of reproductive phase that could decrease rice grain yield and its components, by increasing the spikelets sterility rate, reducing grain filling period and disrupting the sink activity.

Moreover, when photosynthesis reductions are accelerated leaf senescence is faster, decreasing the sucrose-starch conversion via enzymatic activity and consequently reducing the grain yield and the quality (Bahuguna et al., 2017; Chaturvedi et al., 2017; You et al., 2017). Considering that starch represents 80-90% of final grain weight, the events involving since grain filling and final yield are associated to assimilates supplied by current photosynthesis and stem NSC remobilization capacity (Yoshida, 1981). During the vegetative phase and until heading, carbon assimilates are partially and temporally stored in stems and leaf sheaths in rice plants as NSC, which are sinked for all events involving since reproductive organ formation until grain development and maturation phase (Morita & Nakano, 2011; Zhang et al., 2016). For rice, there are studies showing that the stem NSC contribution to grain yield must reach 28% (Pan et al., 2011; Yoshida, 1981). Besides, there are indications that stem NSC at full heading stage of rice can increase the grain ripening ratio in those plants submitted to heat stress, increasing its grain yield stability (Morita & Nakano, 2011). For those plants subjected to drought, pre-anthesis stem NSC accumulation have highlighted its potential to buffer grain-filling in both wheat and rice (Saint Pierre et al., 2010; Yang et al., 2001) and also has been associated to tolerance to long-term partial submergence in rice (Kato et al., 2014).

Thus, the current investigation was carry out aiming to evaluate the effects of high night temperature on NSC remobilization, photosynthesis performance and some yield components in two contrasting rice genotypes for heat tolerance.

2. Material and Methods

2.1 Plant Materials

Two rice genotypes, a heat-tolerant, Nagina22 (N22) (Jagadish et al., 2010), and heat-sensitive, BRS Querência (Quer), based in our previous trials, were evaluated in this study. These genotypes have similar life cycles but significant differences in grain weight when grown under field conditions in previews trials conducted by Embrapa's rice breeding program.

2.2 Growth Conditions and Temperature Treatments

Rice plants were grown in plastic pots (3.0 kg soil) in a greenhouse situated at Embrapa Temperate Climate. The plants were kept in the greenhouse from sowing procedures until plant heading stage (Figure 1A). Thereafter, half of plants from each genotype were taken to two growth chambers (Figure 1B) aiming to impose the temperature treatments. The growth chambers were set to maintain a temperature of 22.0/30±0.5 °C (night/day) for a control treatment and 28.0/30±0.5 °C (night/day), representing a high nighttime temperature (HNT). The photoperiod used was, 10hrs in the dark period and 14hrs light period at 500 µmol m⁻² s⁻¹ approximately.



Figure 1. Installations as facilities used for trials: greenhouse (A) and growth chambers (B)

Four biological replicates of each treatment were carry out considering the required time for these gas exchange and chlorophyll analyzes. In order to ensure that the same sample was ever used for each time point analysis of gas exchange and chlorophyll fluorescence, the younger and totally expanded leaf was labeled. Gas exchange analyzes were carry out at seventh, fourteenth and twenty-first days after treatment imposing; whereas chlorophyll fluorescence measurements were conducted on twenty-first days during the stress period.

Biochemical analyzes were performed in plants submitted to control and stress conditions (HNT), which were collected at two time-points: on seventh days after heading (R_3) and at milk stage (R_5/R_6) (Counce et al., 2000).

2.3 Gas Exchange and Chlorophyll Fluorescence Measurements

At seventh, fourteenth and twenty-first days after stress imposing, the net assimilation rate (P_n) and stomatal conductance (g_s) were quantified between 09:00 and 11:00 h under artificial photosynthetic photon flux (PPF) ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using a portable photosynthesis system infrared gas analyzer (LI-COR 6400XT, LI-COR Biosciences, Lincoln, NE, USA). The measurements were performed using a CO_2 cartridge and a 6400-01 CO_2 Injector System controlled the CO_2 partial pressure entering the cuvette (Ca , μbar) maintaining them in 400 ppm. For all treatments, measurements started on youngest expanded leaf on four different plants ($n = 4$). The mid to distal portion of each leaf blade was inserted in the leaf chamber for gas-exchange measurements. For these parameters measurements, a leaf was acclimated in the light level ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 40 min before gas exchange analyses. Chlorophyll fluorescence analyzes were done using a PAM-2500 fluorometer (Walz Heinz GmbH, Effeltrich, Germany). Before measurements, plants were dark-adapted overnight within the grow chamber. The basal fluorescence (F_0) at open photosystem II (PSII) centers was determined by measuring light, whereas the maximum fluorescence (F_m) at closed or in a reduced state of PSII centers was evaluated after a application of a 0.8 s pulse of saturating light ($7000 \mu\text{mol m}^{-2} \text{s}^{-1}$). The maximum quantum efficiency of PSII (F_v/F_m) was defined as $(F_m - F_0)/F_m$. During measurements the actinic light (red light) was turn on to quantify the steady-state of chlorophyll fluorescence (F_s). Plants in the light-adapted state, F'_m was analyzed via application a saturating pulse, whereas F'_0 was evaluated by switching off the actinic light for 2 s after the saturating pulse and turn on the far-red light. Non-photochemical quenching (NPQ) quantified by ratio $F_m/F'_m - 1$; effective quantum efficiency of PSII Y(II) defined as $(F'_m - F_s)/F'_m$; photochemical quenching (qP) defined as $1 - (F_s - F'_0)/(F'_m - F'_0)$; electron transfer rate (ETR) defined as $\text{PAR} \cdot \text{ETR-factor} \cdot P_{\text{PS2}}/P_{\text{PPS}} \cdot Y(\text{II})$ (Baker 2008).

2.4 Non-structural Carbohydrates Determination (NSC)

Plants from both genotypes and temperature regime were cut in the basal portion of the stem between 09:00 and 10:00 h at seventh day after heading and at milk stage aiming quantify its NSC (total soluble sugar and starch in glucose). Five plants from each treatment were sampled for biochemical analyzes; this plants were separated into leaves, stem and panicles and immediately weighted and dried at 60°C and subsequently stored. Thereafter, the samples were grounded (50 mesh); the extraction done in ethanol 80%, in water bath at 95°C during 10 minutes, centrifuged at 2,500 rpm by five minutes. Total soluble sugar (TSS) determinations were carry out using supernatant extract and the starch was quantified in the residue remaining after soluble sugars extraction; the total sugars were extracted using phenol-acid and the starch in residue remaining, which was dried and further extracted using enzymatic hydrolyze and subsequently quantified via PGO enzyme addition (Chow &

Landhausser, 2004). Glucose, fructose and sucrose determinations were carry out via gas chromatography (Streeter & Strimbu, 1998); the derivatization was done using HMKS:TMCS (3:1).

2.5 Yield Components Measurements

After the stress period, all genotypes were taken back to the greenhouse where were maintained until physiological maturity. After, some yield components were quantified harvesting each plant separately; the grain weight was adjusted to 14% moisture content; were quantified the panicle grain number, percentage of spikelet sterility, 10-panicle weight and 1000-grain weight.

2.6 Statistical Procedures

The homogeneity of variances was tested by the Bartlett test and Normality of data via Shapiro-Wilk test; subsequently data were subjected to analysis of variance (ANOVA). The Least Significant Difference (LSD) among the means was statistically analyzed using Student-Newman-Keuls method ($p < 0.05$). Additionally, when interaction effects were significant, unfold statistical procedures were done aiming to quantify the effects of treatments within each ambient temperature and comparisons for both genetic background within each temperature regime (control and under heat stress).

3. Results

In general, taking into account the values quantified in the two genotypes submitted to temperature regimes become evident that there were not significant differences in their CO₂ assimilate rate and stomatal conductance. However, except for photosynthesis under control conditions on seventh and twenty-first days after heading phase when Quer showed the highest quantified values (Figure 2).

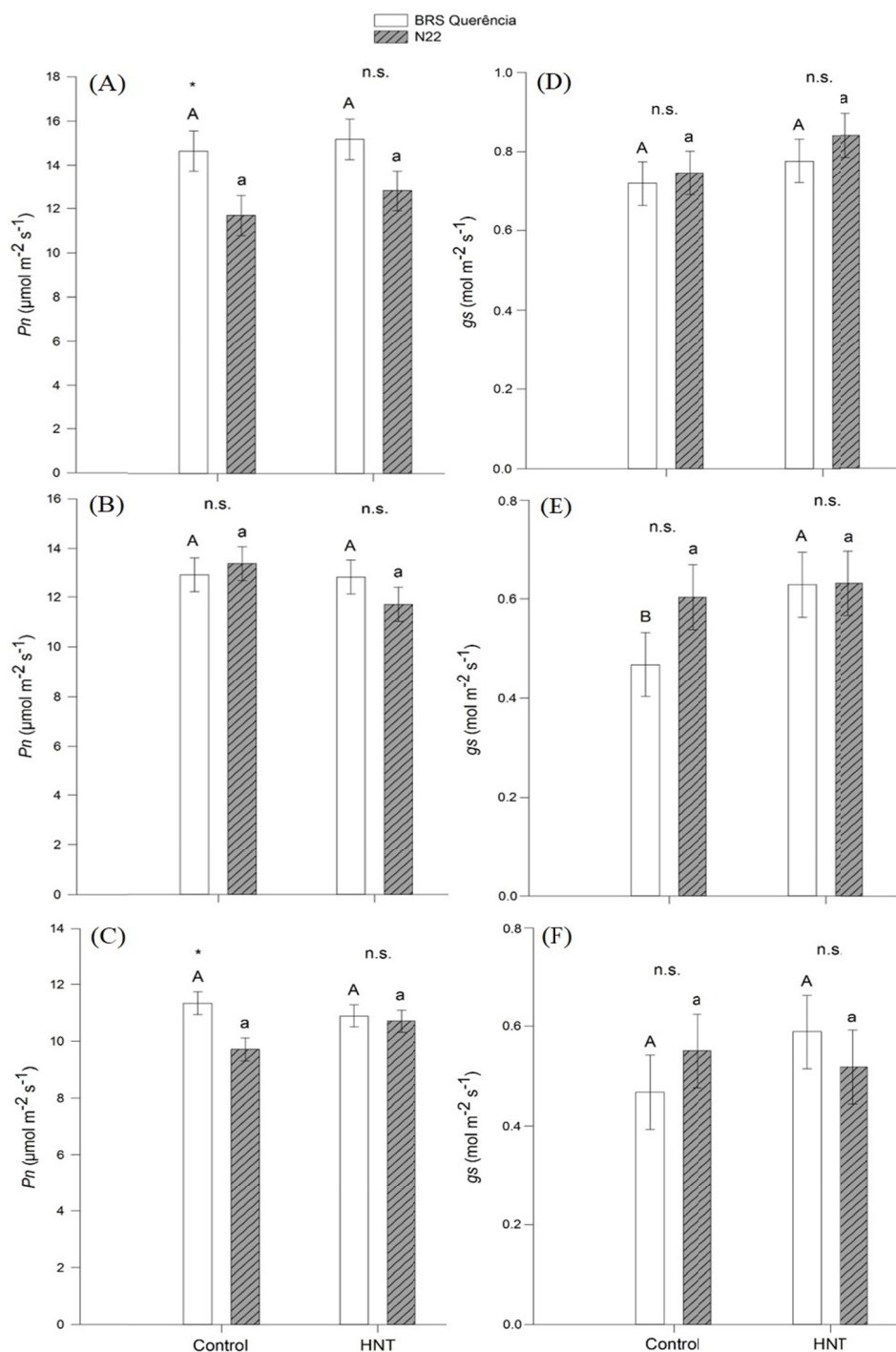


Figure 2. Net assimilation rate (P_n) on seventh (A), fourteenth (B) and twenty-first days after the high night temperatures imposing (C); and stomatal conductance (g_s) at seventh (D), fourteenth (E) and twenty-first days after temperatures regimes imposing (F) in rice genotypes

Note. For white columns (BRS Querência - Quer) and cross gray columns (Nagina22 - N22) followed by the same capital letter and the same lowercase letter, respectively, are not significantly different at the $p < 0.05$ level by the Student-Newman-Keuls test. The symbols * and n.s. mean significant difference at the $p < 0.05$ and non-significant level, respectively, for a given night temperature condition among the contrasting genotypes.

At milk stage, evaluation of the chlorophyll fluorescence were used to complement those shown by gas exchange analysis. Chlorophyll fluorescence can provide information about the functional state of the thylakoid membranes into the chloroplasts when plants are submitted to biotic or abiotic stresses. In responses, changes in

characteristics of fluorescence signals can be quantified in leaves. In our evaluations, when submitted to HNT N22 showed higher values for F_0 (11%) and NPQ (12%) but decreased values for F_v/F_m (4%) and $Y(II)$ (13%) when compared to Quer (Figure 3).

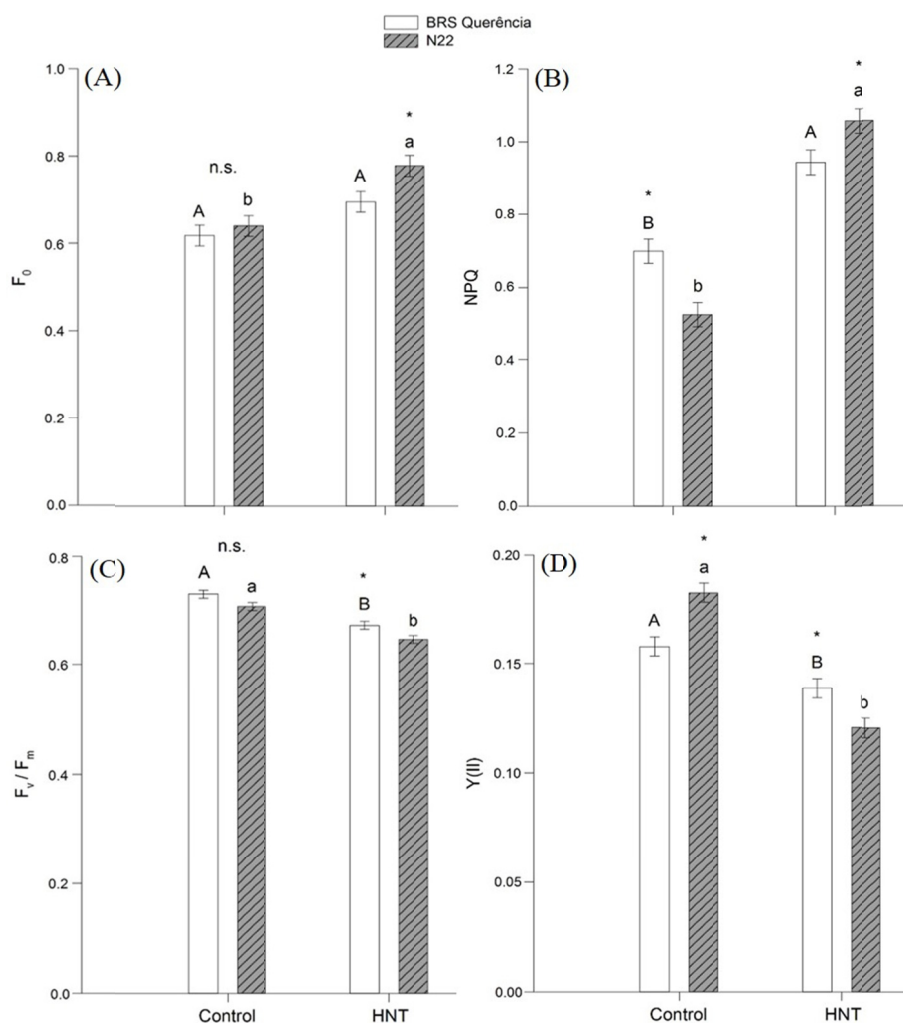


Figure 3. Basal fluorescence - F_0 (A), non-photochemical quenching - NPQ (B), maximum quantum efficiency of PSII - F_v/F_m (C) and effective quantum efficiency of PSII - $Y(II)$ (D) at twenty-first day after temperature regime imposing

Note. For white columns (BRS Querência - Quer) and cross gray columns (Nagina22 - N22) followed by the same capital letter and the same lowercase letter, respectively, are not significantly different at the $p < 0.05$ value by the Student-Newman-Keuls test. The symbols * and *n.s.* mean significant difference at the $p < 0.05$ and non-significant level, respectively, for a given night temperature condition among the contrasting genotypes.

For TSS on seventh and twenty-first days and for starch content on twenty-first there were a significant temperature regimes \times genotypes interaction ($P < 0.001$) (Figure 4).

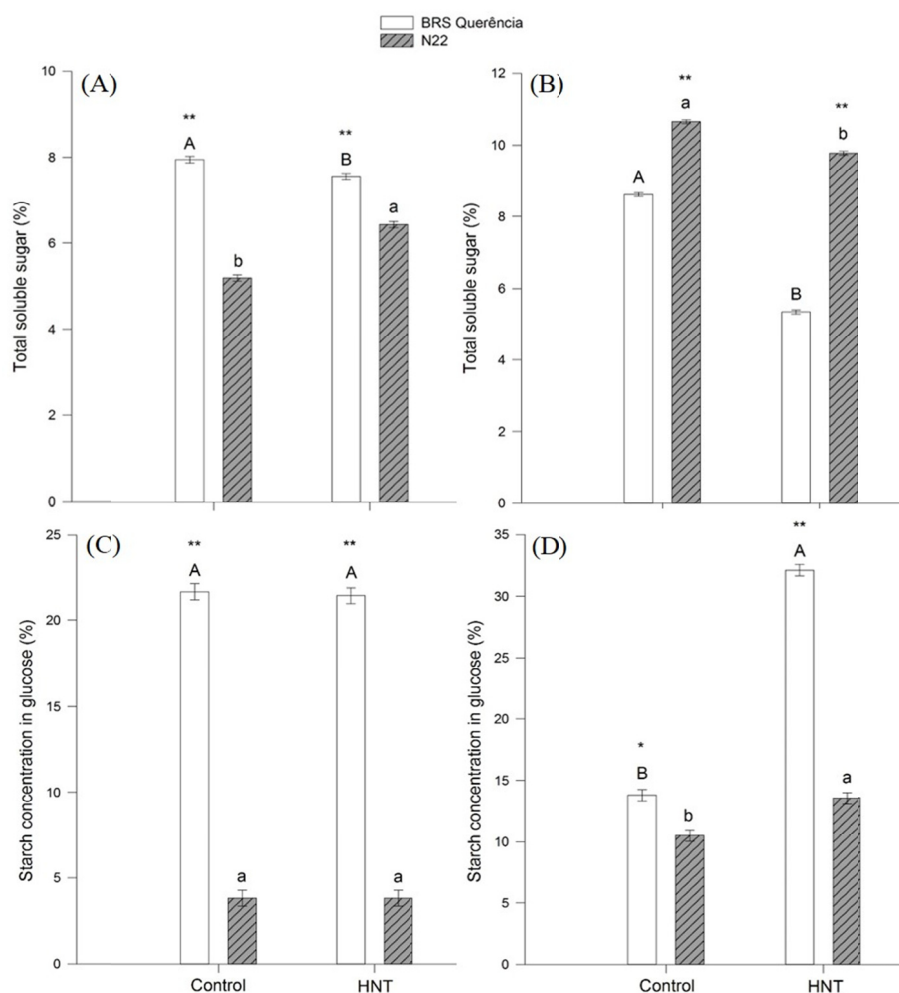


Figure 4. Total soluble sugar at seven (A) and twenty-one days after the imposition of nighttime temperatures (B); and starch concentration in glucose on seventh (C) and twenty-first days after the temperature regime imposing (D)

Note. For white columns (BRS Querência - Quer) and cross gray columns (Nagina22 - N22) followed by the same capital letter and the same lowercase letter, respectively, are not significantly different at the $p < 0.05$ level by the Student-Newman-Keuls test. The symbols *, ** and *n.s.* means significant difference at the $p < 0.05$; $p < 0.01$ and non-significant level, respectively, for a given treatment condition among the contrasting genotypes.

Quer genotype showed the highest TSS and starch values on seventh day after stress imposing, independently of treatments. When analyzing the tendency of two genotypes under different temperature regime were observed that under HNT, the TSS content decreased (5%) and increased at (23%) for Quer and N22, respectively. In same way, the starch content for Quer genotype were higher (563%) when compared to N22 on seventh day independently of temperature regime, whereas values shown by N22 were always lower and unaltered across treatments. During this period, sucrose and glucose were higher for Quer genotype independently of treatment; whereas for N22 there was an increase in fructose level (30%) from control to HNT (Figure 5).

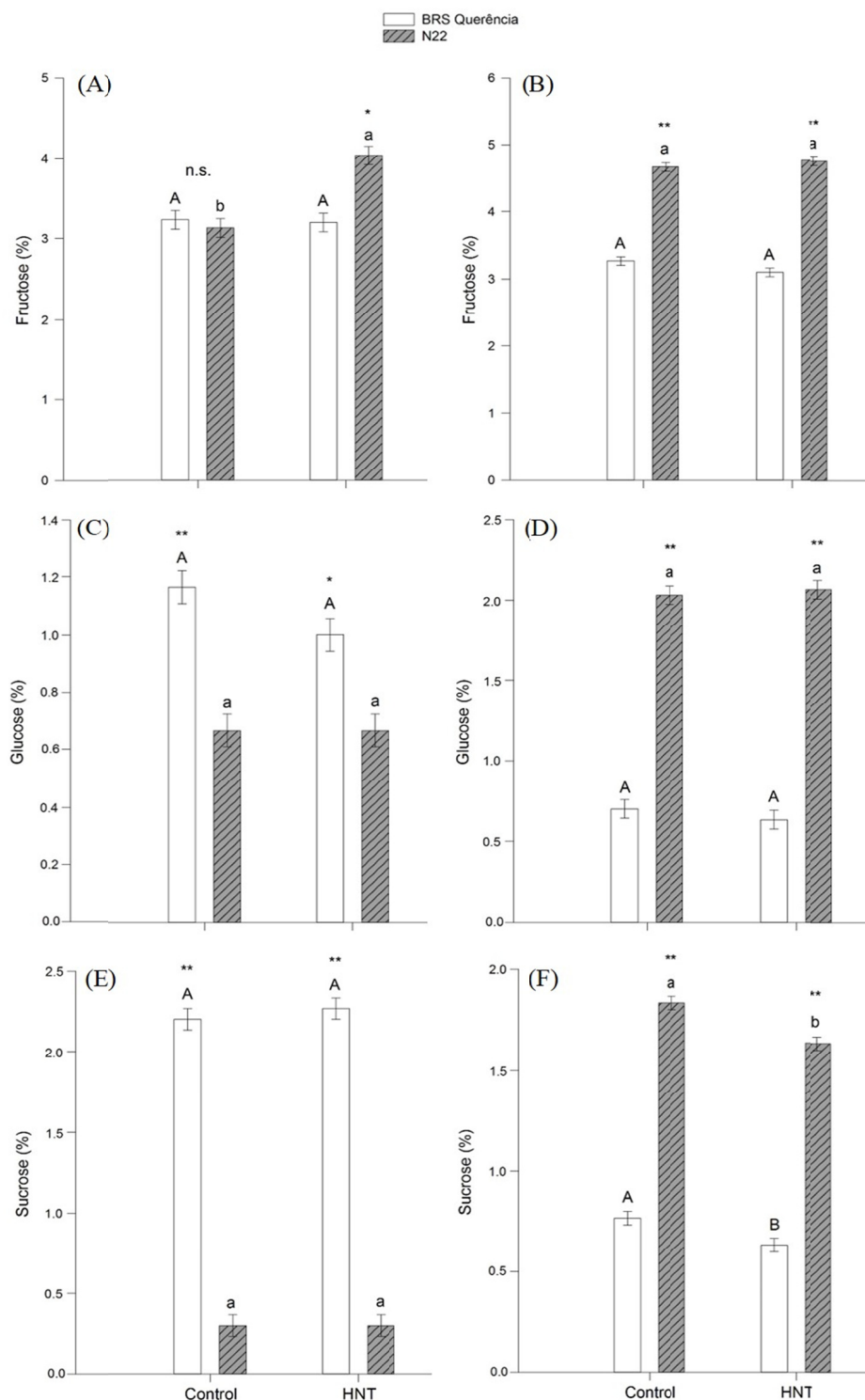


Figure 5. Fructose on seventh (A) and on twenty-first days after treatment imposing (B); glucose on seventh (C) and twenty-first days after treatment imposing (D); and sucrose at seventh (E) and twenty-first days after treatment imposing (F)

Note. For white columns (BRS Querência - Quer) and cross gray columns (Nagina22 - N22) followed by the same capital letter and the same lowercase letter, respectively, are not significantly different at the $p < 0.05$ level by the Student-Newman-Keuls test. The symbols *, ** and *n.s.* mean significant difference at the $p < 0.05$; $p < 0.01$ and non-significant level, respectively, for a given night temperature condition among the contrasting genotypes.

At milk stage, it was also confirmed significant HNT \times genotypes interaction ($P < 0.001$) for TSS and starch (Figure 4). The TSS content was higher in N22 as compared to Quer genotype, independently of imposed treatments. Besides, N22 showed a lower decrease ratio in TSS from control to HNT conditions (8%) whereas a more abrupt reduction (38%) were highlighted for Quer at this phase. In other way, higher levels of starch were maintained in the Quer in both temperature regimes; additionally, there were a stronger starch accumulation (increase of 132%) from control to HNT for this genotype whether compared to N22. At milk stage, there was an increase of 55% for fructose, 350% for glucose and 266% for sucrose in N22 when compared to Quer genotype. Besides, both genotypes maintained unaltered the fructose and glucose levels from control to HNT regimes, except for sucrose content where were highlighted significant decreases for both evaluated genotypes (Figure 5).

For yield parameters, neither 10-panicles weight nor grain per panicle and spikelet sterility showed significant HNT \times genotypes interaction. However, for 1000-grain weight was verified significant ($P < 0.001$) interaction (Figure 6). Additionally, whereas the HNT decreased grain weight for Quer, unaltered performance was highlight for N22, indicating its higher stability when compared ones.

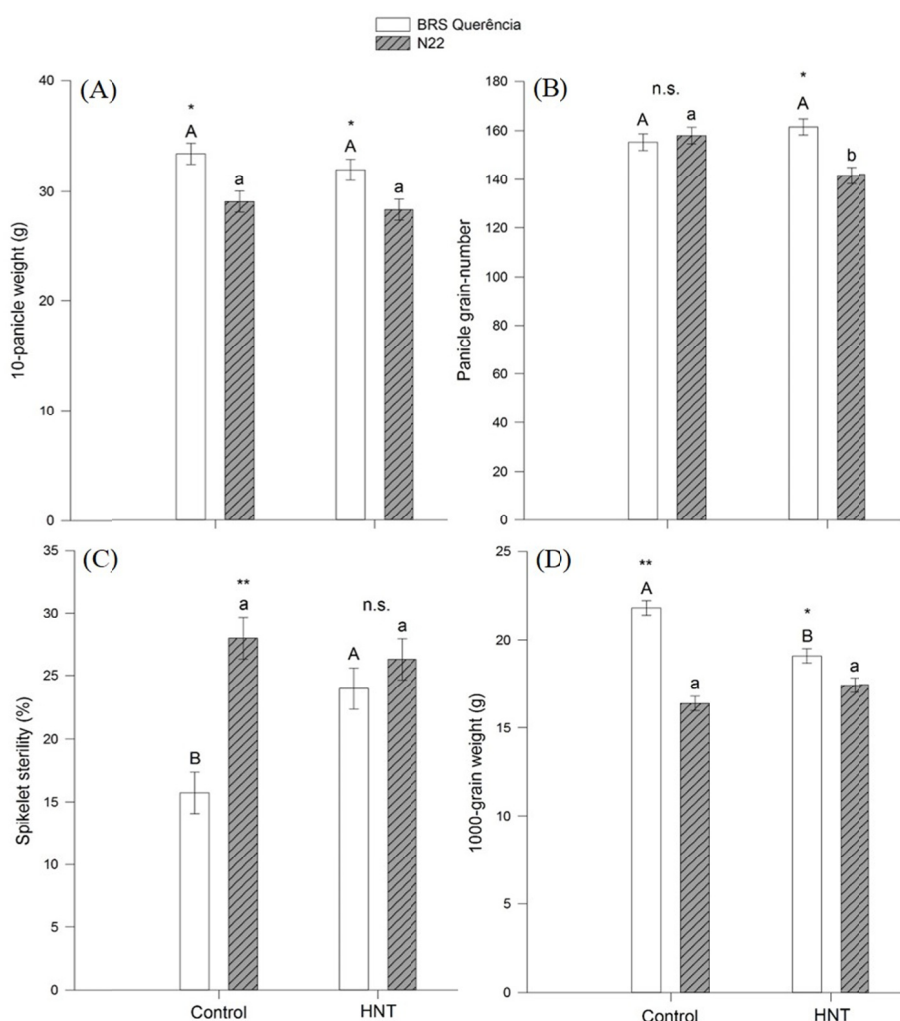


Figure 6. 10-panicle weight (A), panicle grain-number (B), spikelet sterility (C) and 1000-grain weight (D) in rice genotypes submitted to different temperatures regime

Note. For white columns (BRS Querência - Quer) and cross gray columns (Nagina22 - N22) followed by the same capital letter and the same lowercase letter, respectively, are not significantly different at the $p < 0.05$ level by the Student-Newman-Keuls test. The symbols *, ** and *n.s.* mean significant difference at the $p < 0.05$; $p < 0.01$ and non-significant level, respectively, for a given night temperature condition among the contrasting genotypes.

4. Discussion

In this study, our results indicate that N22 maintain unaltered yield components such as spikelet sterility rate, grain per panicle and 1000-grain weight when submitted to high night temperature. On the other hand, Quer genotype showed an increase of 8% and a decrease of 12% for spikelet sterility percentage and 1000-grain weight, respectively. The endosperm cell of rice contributes for more than 90% of the grain weight of a caryopsis. Thus, the size of sink and/or low activity of enzymes involved in the carbohydrates metabolism could contribute to a low grain filling rate depending of their degree of sensibility to HNT. In this study, under optimum HNT the Quer genotype, which have a bigger panicle when compared to N22, showing a more sink strength with a decrease of 36% of stem starch concentration from heading to milk stage; in other way, when submitted to HNT its starch levels were unaltered during evaluated period. Results of different studies have indicated that enzyme activities involved in the breakdown of sucrose in the sink could influence the sink activity and, consequently define the local concentration sucrose-starch rate, as result of unloading of sucrose from phloem (Liang et al., 2001; Ranwala & Miller, 1998). Besides, there is need to consider that SuSase is a main enzyme involved in the cleavage of sucrose in rice grains; because its importance in this event it have been considered as an important biochemical marker of sink activity (Kato, 1995; Liang et al., 2001; You et al., 2016; Zhang et al., 2014). In other way, AGPase has been also considered as a key enzyme participating in the starch synthesis, and its activity is associated with rate and quantity of starch synthesis (Ahmadi & Baker, 2001; Yang et al., 2004; Yang et al., 2017). AGPase at the early grain filling stage were decreased as result of supra-optimal temperatures (Ahmed et al., 2015); under other abiotic stress, as such water deficit, SuSase and AGPase had its activities levels influenced. Our results suggest that the bigger capacity of N22 to maintain higher soluble sugar levels (fructose, glucose and sucrose) at milk stage can have contributed to their better yield component stability across night temperatures imposed.

Interestingly, even under HNT the evaluated genotypes showed similarity in some gas exchange and chlorophyll fluorescence performance *e.g.* P_n , g_s , F_0 , F_m , F_v/F_m , YII, NPQ, qP and ETR, suggesting unchanged photosystem II photochemistry, linear electron flux, and CO₂ assimilation, indicating that photosynthesis machinery was not the limiting factor for lower TSC and starch levels found in stem of N22 on seventy day after heading stage when compared to Quer. On the other hand, its higher sink strength when submitted to HNT can originated from its heat tolerance characteristics as reported by different authors (Mutum et al., 2016; Poli et al., 2013; Prasanth et al., 2016). Additionally, was highlighted that N22 had the strongest sink associated to accelerated senescence during stress imposing period.

Studies involving the approaches that aim to quantify the heat-stress effects on rice physiological performance and their yield components and identification of genetic variability have become increasingly in last decade. However, few studies have concentrate efforts to evaluate rice biochemical/physiological responses under high night temperatures (Chaturvedi et al., 2017) and there are scarcity of information about rice plant responses when are submitted to high night supra-optimal temperatures. Different studies have suggested that the amount of NSC mobilization in the post-heading stem could result from sink strength and environment/management (Chen & Wang, 2008; Kim et al., 2011; Li et al., 2017; Morita & Nakano, 2011; Wada et al., 2017; Yang et al., 2000). In other way, NSC level at maturity cannot be attributed to lower mobilization because additional photoassimilate re-accumulation can be result from its semi-perennial characteristics. Besides, accumulation, remobilization and re-accumulation of NSC in stem can be influenced by genotype, environment and genotypes environment interaction. In this sense, in the next step efforts will be concentrating to monitor NSC dynamics from heading until maturity phase in different organs, *i.e.* leaves, stem and spikelets in development aiming to elucidate these responses and define the more adequate organ and best phase to validate this approach as proxy for rice physiological breeding.

Accumulation and mobilization of NSC in plant's rice are mediated by various enzymes, which include catalysts of starch synthesis/breakdown and sucrose synthesis/breakdown or proteins responsible by its transport across organs. Different reports indicate the role of enzymes such as α -amilase, AGPase, BE, SSS, GBSS, plastidial FBPase, and sucrose synthase, *i.e.* Susy and Susase in the accumulation and remobilization of NSC in rice (Fu et al., 2011; Ishimaru et al., 2004; You et al., 2017). Considering the complexities o stem NSC dynamics and the underlying events culminating to carbohydrate allocation and its utilization, lead us to think that in the future, NSCs oscillation probably will be serve as proxy for sink strength selection criterion rather than a direct target for breeding under climate change scenarios. Summarizing, rice plants can be affected by complex genome x environment x management interactions which results in phenotypic plasticity as a result of the variability of genetic components. Whether by on side, recently advances have been made in genetic analysis, propitiated by development of new approaches as such CRISPR/Cas (Cong et al., 2013) and base editor (Liang et al., 2017), on

the other hand, in respect to non-invasive physiological phenotyping, there are scarcity of large-scale analyses of the underlying physiological mechanisms that plants trigger in response to environmental stimulus. In this sense, even in an initial phase, our data indicate that external phenotype such as spikelet sterility and 1000-grain weight is determined by the sum of the complex interactions resultant of an internal, physiological, and biochemical phenotype. A high-dimensional physiological phenotyping across scales is needed that integrates the precise characterization of the internal phenotype into high-throughput phenotyping of whole plants and canopies (Großkinsky et al., 2015).

In conclusion, the results indicate that high night temperature imposed from heading to milk stage can leading changes in source-flow-sink related attributes in contrasting genotype, leading to changes in nature of NSC accumulation and translocation. These results suggest yet that higher translocation capacity shown by N22 can contribute to their lower spikelet sterility rate and higher 1000-grain weight stability across the environments tested. Moreover, indicate that selecting genotypes with higher capacity to stem NSC translocation at HNT could lead to more grain yield stability especially for those regions where occurrence of high night temperature during critical reproductive phase is common.

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