Relationship between root exudation of organic carbon and physiological variables of irrigated rice cultivars

Janielly Silva Costa Moscôsou, Leandro Souza da Silva, Camila Peligrinotti Tarouco, Fernando Teixeira Nicoloso, Fabiane Figueiredo Severo, Natália Tobin Aita, Luís Henrique Ferreira Prigol

ABSTRACT: Rhizospheric carbon resulting from root exudation is one of the substrates used by the soil microbiota, and reflects methane (CH₄) emissions in anoxic environments such as irrigated rice cultivation. With the increase of the photosynthetic capacity of the plant in the reproductive period, there is greater accumulation of biomass which, in turn, increases the rate of root exudation. However, genotypic variations in the physiological aspects of rice plants may be related to the amount of root exudates. Ten cultivars of irrigated rice were evaluated for the exudation rate of total organic carbon (EXRToc), shoot dry matter (SDM), and physiological variables related to photosynthesis during the full flowering (blooming) period. Two experiments were conducted in the greenhouse of the Department of Soils of the UFSM (University of Santa Maria, Santa Maria, RS, Brazil) in a completely randomized experimental design. The cultivars presented significant differences in EXRToc, SDM, and all physiological variables as well as positive and significant correlations between EXRToc and physiological variables. Early cultivars were more inefficient in the physiological variables reflecting reduced values of EXRToc and SDM whereas medium-cycle cultivars were more efficient with larger EXRToc and SDM reflections.

Key words: Oryza sativa, photosynthesis, exudates, methanogenesis.

INTRODUCTION

Rhizospheric carbon resulting from root exudation is one of the main substrates used by the soil microbiota, reflecting carbon dioxide (CO₂) emissions in aerobic and methane (CH₄) in anoxic environments such as irrigated rice cultivation (GARCIA-ORENES et al., 2010). As a result of the increase in the photosynthetic capacity of the plant during the reproductive period, there is greater accumulation of biomass which, in turn, boosts the increase in rhizodeposition, influencing the rhizospheric carbon dynamics (BATATAChARYYA et al., 2013). Several studies have reported intense
methane emission peaks in areas of irrigated rice cultivation coinciding with plants flowering period. These findings show the contribution of exuded carbon compounds as a substrate for the generation of greenhouse gases (ITOH et al. al., 2011; SU et al., 2015). The microorganisms present in the anoxic environments, mainly the methanogenic bacteria, use carbon from root exudates as final acceptors of electrons in their electron transport chain which result in the production of CH₄.

The genetic variability found among rice traditional cultivars or among those developed by breeding programs, of medium-cycle (medium duration cultivar) or early-cycle (short duration cultivar), may interfere with the amount of photo-assimilated carbon that is translocated to the plants root and then exuded in the form of organic compounds containing carbon. This variability should be reflected in different physiological variables that are associated with the photosynthetic activity of the plant which should also be closely related to root exudation. The knowledge of this relationship may help in the selection of materials to be cultivated and in rice breeding programs according to the goal of the selection of the materials.

Photosynthesis is the physiological process by which plants capture solar energy of and convert it into chemical energy. This process involves oxidation reactions in which water is oxidized with the release of O₂ and CO₂ is reduced for carbon fixation and formation of organic compounds (SOUZA, 2016). However, photosynthesis is closely related to transpiration through the stomata. At the same time that the stomata offer resistance to the diffusion of water vapor from the leaf into the atmosphere, these pores also constitute a barrier to CO₂ capture (PEARCY & PTISTISCH, 1991).

Genotypic variations in the photosynthetic metabolism of rice plants such as the rate of CO₂ net assimilation, stomatal conductance, intercellular CO₂ concentration, and transpiratory rate have been reported in the literature (YOSHIDA et al. al., 1976; GU et al., 2012; LORENÇONI, 2014). Therefore, it is important to understand how the morphophysiological variables of different irrigated rice cultivars may be related to the supply of root exudates that function as a source for the methanogenesis process in rice cultivation under the flood irrigation regime.

The present study aimed to quantify the total organic carbon exudation rate of irrigated rice cultivars during the full flowering period and to correlate this exudation with physiological variables of each cultivar.

MATERIALS AND METHODS

Two experiments were carried out at the greenhouse of the Department of Soils (DS) of the Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. In the first experiment, 10 cultivars of irrigated rice were cultivated in nutrient solution (FURLANI & FURLANI, 1988), 6 early cycle cultivars (‘BRS Quêrcia’, ‘Embrapa 130’, ‘IRGA 421’, ‘Koshihikari’, ‘BRS Pampa’ and ‘IRGA 430’) and 4 medium cycle cultivars (‘BRS Taim’, ‘IAS Formosa’, ‘IRGA 422’, ‘IRGA 428’). Vessels with a capacity of 7L containing 6L of nutrient solution were conducted in a completely randomized experimental design with three replicates.

Plants were individually grown until the full flowering period of each cultivar, when the concentrations of total organic carbon (TOC) of root exudates were quantified. In this procedure, which was based on the method adapted from AULAKH et al. (2001), roots were washed with distilled water and each plant was placed in glass bottles (containing 600mL of CaSO₄ solution at 0.01mol L⁻¹ - collection medium) so that the roots of plants remained completely submerged in the collection medium for a period of 2 hours. Immediately after collection, each sample from the collection medium was routed to the TOC-LCPH/CPN total organic carbon analyzer apparatus, discounting any values of the non-plant collection medium functioning as a blank.

The rates of TOC exudation released by roots of rice cultivars were calculated by equation 1:

\[
\text{EXR}_{\text{TOC}} = \frac{C_{\text{exp}} - C_{c} \times 600 \times 24}{PC}
\]

In which: \( \text{EXR}_{\text{TOC}} \) is the rate of TOC exudation released by roots of irrigated rice cultivars (mg C plant⁻¹ day⁻¹), \( C_{\text{exp}} \) is the exuded carbon per plant (mg mL⁻¹), \( C_{c} \) is the carbon of the control collection medium, 600 is the amount of collection medium used for each plant (mL plant⁻¹), 24 is the number of hours of the day, and PC is the collection period of the exudates (2 hours).

The second experiment with the same irrigated rice cultivars was performed in vessels with a capacity of 9L containing 7kg of a typical hydromorphic haplic planosol soil (EMBRAPA, 2013). Soil was sampled in the experimental field of DS of the UFSM, previously air dried, slashed, and sieved in a 4mm mesh with the cultivation of 6 rice plants per vessel in a completely randomized experimental design with three replicates. Vessels remained under continuous flooding until the...
maturation stage of the cultivars and were then fertilized according to the recommendations proposed by SOSBAI (2016).

Physiological evaluations were carried out using the “Infra Red Gas Analyzer - IRGA” model LI-6400XP, LI-COR, in two phenological phases, according to the scale published by COUNCE et al. (2000). The first reading was carried out when the early cultivars were in the full bloom stage (anthesis) and the medium-cycle cultivars were in the panicle exertion phase. The second reading was carried out when the early-cycle cultivars were in the physiological maturation stage and the medium-cycle cultivars were mostly in the anthesis stage. Readings were carried out in two plants per vessel, in the middle third of the fully expanded flag leaf of the stem, with a photosynthetic irradiance of 1500μmol m\(^{-2}\) s\(^{-1}\) and CO\(_2\) concentration of 400μmol mol\(^{-1}\). We measured the net assimilation rate of CO\(_2\) (A - μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), the stomatal conductance of water vapors (Gs - mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), the transpiration rate (E - mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), the intercellular CO\(_2\) concentration (Ci - μmol m\(^{-2}\) s\(^{-1}\)). From these, it was possible to calculate the water use efficiency (WUE - mol CO\(_2\) mol H\(_2\)O\(^{-1}\)) obtained by the ratio between the amount of CO\(_2\) fixed by photosynthesis and the amount of transpired water, and the instantaneous efficiency of carboxylation by Rubisco (A/Ci) obtained by the relation between the amount of CO\(_2\) fixed by photosynthesis and the intercellular CO\(_2\) concentration. At the end of the experiment, the dry matter of the aerial part of each cultivar was quantified after drying in a greenhouse with forced air ventilation at 65°C.

The means and standard deviation (SD) of the data were calculated, and analysis of variance and F test (P<0.05) were performed with a subsequent Scott-Knot test (P<0.05) as well as phenotypic and genotypic correlations between EXRToc and physiological variables. Phenotypic correlations were estimated by t-test, and the genotypic correlations were estimated by “bootstrap” with 5000 simulations. Correlations were classified according to SHIMAKURA & RIBEIRO JUNIOR (2012) as: 0.0 to 0.19 (very weak), 0.20 to 0.39 (weak), 0.40 to 0.69 (moderate), 0.70 to 0.89 (strong), and 0.90 to 1.00 (very strong). We used the computer program (software) in genetics and statistics Genes in the statistical procedures (CRUZ, 2006).

RESULTS AND DISCUSSION

The cultivars presented significant differences in the variables evaluated in the two experiments, carbon exudation rate, shoot dry matter quantification and in all physiological variables in the two readings. The highest rates of carbon exudation were reported in the cultivars BRS Taim, BRS Pampa, and IRGA 428, and among the lowest were the cultivars Koshihikari, IRGA 421 and Embrapa 130 (Table 1). In general, the cultivars of early cycle were those that obtained lower rates of exudation. This is probably due to the fact that these cultivars have a lower development cycle and have lower dry matter production. The longer the cultivars remain in the field, the higher the photosynthetic rates are obtained, resulting in a higher accumulation of biomass, greater translocation of carbon to the root, and greater rhizosynthetic rates in the soil (BHATTACHARYYA et al., 2013).

The cultivars ‘BRS Pampa’ and ‘IRGA 428’ also obtained the highest values of net CO\(_2\) assimilation rate in the 1st reading, while the cultivar ‘IRGA 421’ presented the lowest value. In the 2nd reading, the cultivars ‘Embrapa 130’, ‘BRS Taim’ and ‘BRS Pampa’ presented the highest values, whereas the cultivar ‘IRGA 421’ continued to present the lowest value. These results may be associated with root exudation as cultivars ‘BRS Taim’ and ‘IRGA 428’ (both medium cycle cultivars) and ‘BRS Pampa’ were the ones that had the highest exudation rate, highest rates of assimilation of CO\(_2\), and highest values of dry matter as well as early-stage ‘IRGA 421’ which presented the lowest values for these variables (Table 1).

The highest values of stomatal conductance were observed in the cultivars ‘IRGA 428’ and ‘BRS Pampa’ (1st and 2nd reading) and the cultivar ‘Embrapa 130’ (2nd reading). The lowest results were observed in the cultivars ‘IAS Formosa’ and IRGA 421 (both readings) and in the cultivar ‘Koshihikari’ (2nd reading). High stomatal conductance is possible in situations of abundant water availability as in the case of irrigated rice cultivation. Although, transpiration is elevated under these conditions, it allows a higher rate of CO\(_2\) absorption ensuring its continuous fixation during photosynthesis (TAIZ et al., 2017). This higher CO\(_2\) fixation may increase the amount of carbon to be translocated to the roots. Considering that the cultivars of rice were being maintained with the same irrigation and were under the same environment, the observed differences are inherent characteristics of each cultivar as in the cultivars ‘BRS Pampa’ and ‘IRGA 428’ that presented high stomatal conductance and high net assimilation rates of CO\(_2\) which provides high rates of root exudation.

In general, the cultivars that presented the highest rates of stomatal conductance and liquid CO\(_2\)
assimilation were those with the lowest concentration of intercellular CO$_2$ and vice versa (Table 1). Apparently these physiological differences between the cultivars showed the presence of possible variations among the cultivars regarding photosynthetic CO$_2$ utilization. This means that the assimilated CO$_2$ is being consumed to form the final photosynthesis products such as sucrose or starch or even exudates, thus reducing intercellular CO$_2$ concentration values and indicating a high photosynthetic CO$_2$ utilization.

With regard to the transpiration rate, the ‘BRS Pampa’ cultivar presented the highest value in both readings whereas the ‘IRGA 421’ the lowest one together with the cultivar ‘Embrapa 130’ in the 1$^{st}$ reading, and the cultivars ‘Koshihikari’, ‘IRGA 422’, ‘BRS Querência’ (early cycle), and ‘IAS Formosa’ in the 2$^{nd}$ reading. In general, the transpiration rate values are in agreement with the values of stomatal 

Table 1 - Mean values (n = 30) obtained for EXR$_{Toc}$ in the full flowering stage (nutrient solution experiment) 1$^{st}$ reading (at 78 days after transplant) of the physiological variables (soil experiment) evaluated in 10 cultivars of irrigated rice under greenhouse conditions.

| Treatments       | A    | Gs   | Ci    | E    | WUE  | A/Ci  | EXR$_{Toc}$ |
|------------------|------|------|-------|------|------|-------|-------------|
| BRS Querência    | 24.16d | 1.37b | 307.8e | 11.23b | 2.30b | 0.08c | 54.90c      |
| Embrapa 130      | 23.63d | 0.88d | 313.9d | 8.74d  | 2.35b | 0.08c | 20.72c      |
| IRGA 421         | 15.19e | 0.84d | 343.6a | 8.36d  | 2.01c | 0.04d | 19.80c      |
| Koshihikari      | 24.08d | 1.09c | 327.3c | 9.97c  | 2.28b | 0.07c | 1.56d       |
| BRS Pampa        | 33.13a | 1.41b | 323.5c | 14.13a | 2.35b | 0.10a | 273.90b     |
| IRGA 430         | 23.38d | 1.22c | 329.3c | 10.92b | 2.08c | 0.07c | 33.68c      |
| BRS Taim         | 26.11c | 1.27c | 334.2b | 10.75b | 2.12c | 0.08c | 394.34a     |
| IAS Formosa      | 26.22c | 0.63e | 306.6e | 9.84c  | 2.77a | 0.09b | 54.48c      |
| IRGA 422         | 28.14b | 1.21c | 325.0c | 11.59b | 2.37b | 0.09b | 29.88c      |
| IRGA 428         | 28.07b | 1.61a | 326.7c | 12.18b | 2.18c | 0.09b | 252.10b     |
| General mean     | 25.23 | 1.15  | 323.76 | 10.76  | 2.28  | 0.08  | 126.73      |

Mean values (n = 30) obtained for shoot dry matter and 2$^{nd}$ reading (at 93 days after transplant) of the physiological variables (soil experiment) evaluated in 10 cultivars of irrigated rice under greenhouse conditions.

| Treatments       | A    | Gs   | Ci    | E    | WUE  | A/Ci  | SDM        |
|------------------|------|------|-------|------|------|-------|------------|
| BRS Querência    | 16.11d | 0.96c | 340.80b | 7.72d  | 1.93e | 0.04c | 63.13c     |
| Embrapa 130      | 30.54a | 1.42b | 325.09d | 10.78b | 2.89c | 0.10a | 46.23f     |
| IRGA 421         | 6.80e  | 0.81d | 368.39a | 7.11d  | 1.02g | 0.02d | 53.56e      |
| Koshihikari      | 16.07d | 0.58c | 333.55c | 7.29d  | 2.17d | 0.05c | 56.85d     |
| BRS Pampa        | 28.08b | 1.91a | 335.67c | 11.91a | 2.23d | 0.09a | 69.22a      |
| IRGA 430         | 16.34d | 0.97c | 313.08e | 9.63c  | 1.64f | 0.05c | 61.44c     |
| BRS Taim         | 27.43b | 0.83d | 314.98e | 9.29c  | 3.01b | 0.09a | 66.50b      |
| IAS Formosa      | 16.92d | 0.67e | 323.90d | 7.69d  | 1.98e | 0.05c | 59.24c      |
| IRGA 422         | 20.31c | 1.15c | 339.64b | 7.06d  | 3.33a | 0.06b | 60.58c      |
| IRGA 428         | 21.74c | 1.26b | 340.03b | 9.51c  | 2.20d | 0.06b | 65.21b      |
| General mean     | 20.0  | 1.06  | 333.53 | 8.80  | 2.24  | 0.06  | 60.20       |
| CV (%)           | 5.01  | 4.21  | 0.87   | 8.65  | 2.43  | 3.83  | 19.47       |

A = net assimilation rate of CO$_2$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$), Gs = stomatal conductance (mol H$_2$O m$^{-2}$s$^{-1}$), Ci = concentration of intercellular CO$_2$ (μmol CO$_2$ mol$^{-1}$), E = transpiration rate (mmol H$_2$O m$^{-2}$ s$^{-1}$), WUE = water use efficiency (mol CO$_2$ mol H$_2$O$^{-1}$), A/Ci = instantaneous carboxylation efficiency by Rubisco, EXR$_{Toc}$ = total organic carbon exuded from roots (mg C plant$^{-1}$ day$^{-1}$), SDM = shoot dry matter of (g vessel). Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P <0.05).
conductance. Vegetative transpiration, besides being influenced by environmental or external factors, is also influenced by internal factors conditioned by the morphological and physiological characteristics of the plants (Pupatto, 2003). Reduction in the transpiration rate often results in an increase in water use efficiency since a smaller amount of water is transpired to produce a certain amount of dry matter. This is observed in the cultivar ‘BRS Taim’ (medium cycle cultivar) that reduced its transpiration, thus increasing the efficiency of water use and stimulating a higher rate of carbon exudation.

Regarding to the values of instantaneous carboxylation efficiency by Rubisco observed in the 1st reading, the highest values were found in the cultivars ‘BRS Pampa’, ‘IRGA 428’, ‘IRGA 422’, and ‘IAS Formosa’ whereas the cultivar ‘IRGA 421’ presented the lowest value. Already 2nd reading, the cultivar ‘BRS Pampa’ continued to present one of the highest values for this variable, accompanied by cultivars ‘BRS Taim’ and ‘Embrapa 130’ whereas ‘IRGA 421’ followed with the lowest value obtained. High values of internal CO$_2$ concentration associated with low rates of CO$_2$ net assimilation indicate a decrease in the instantaneous carboxylation efficiency due to the reduced availability of ATP, NADPH, and substrate to Rubisco resulting in the decrease of the activity of this essential enzyme in the CO$_2$ binding process (Silva et al., 2015). This may explain the low efficiency of Rubisco obtained by ‘IRGA 421’ which may have influenced the values of exudation rate and shoot dry matter in this early cycle cultivar. In contrast, the cultivars that presented the highest rate of CO$_2$ net assimilation were those that obtained the lowest concentration of intercellular CO$_2$ and, as a result, higher values of instantaneous efficiency of carboxylation by Rubisco.

As the cultivars had the same condition of luminosity, water availability and mineral nutrition, we may affirm that the obtained differences are actually linked to morphophysiological factors specific to each cultivar.

The physiological variables and the exudation rate, in general, presented genotypic correlations that, if not similar to the phenotypic ones, were higher and of equal sign, indicating that there was no influence of the environment on the expression of the characters and effective experimental precision. This ensures that the correlations and differences presented between the cultivars are expressions linked to morphophysiological factors specific to each cultivar (Table 2).

There was a strong and moderate positive correlation between transpiration rate and exudation rate in the 1st and 2nd readings, respectively, and a strong correlation between stomatal conductance and exudation rate in the 1st reading. This is the result of strong and very strong positive correlations in the two readings between stomatal conductance and transpiration rate (Table 2). Thus, the cultivars with higher transpiration were those that obtained greater stomatal conductance whereas those with lower transpiration had the lowest values for stomatal conductance therefore influencing the exudation rate (see Table 1). Positive correlations were observed between CO$_2$ net assimilation rate and moderate exudation rate in the 1st and 2nd readings (Table 2) which reinforces the hypothesis that high photosynthetic rates are directly related to the exuded carbon of the roots of rice cultivars. It is estimated that 30 to 60% of the photosynthetically fixed carbon in the plant would be translocated to the roots, and a considerable proportion of this carbon (up to 70%) could be released into the rhizosphere by means of rhizodeposition (Yiqi & Zhou, 2010).

Significant correlations were also observed in the physiological variables (Table 2). Positive correlations between the net assimilation rate of CO$_2$ and stomatal conductance under optimal water conditions as in the present study have been observed in rice by Kondo et al. (2000) and Lorençoni (2014). The strong and very strong positive correlations between CO$_2$ net assimilation rate and transpiration rate in both readings indicate the effectiveness of the stomatal adjustment mechanism on the transpiration and CO$_2$ assimilation rates, which are reflected in the water use efficiency and may explain the positive correlations obtained between these variables and the instantaneous efficiency of carboxylation by Rubisco.

For rice, which is a C3 plant, about 400 molecules of water are lost to each carbon molecule fixed by photosynthesis resulting in high water use efficiency for this species. This high value in the C3 plants results from the: concentration gradient governing the water loss which is about 50 times greater than the one that regulates the CO$_2$ influx. CO$_2$ difuses 1.6 times slower by air than water (as the CO$_2$ molecule is larger than the H$_2$O molecule and has a lower diffusion coefficient). CO$_2$ has to cross the plasma membrane, the cytoplasm and the chloroplast envelope before being assimilated into the chloroplast. These membranes increase the resistance of the CO$_2$ diffusion pathway (Taiz et al., 2017).

Based on the results of this study, we were able to detect a response variability among rice cultivars in the use of photosynthetic CO$_2$ due to the physiological differences that these cultivars present.
Such behavior directly influenced the root exudation rates so that these correlations may help explain the behavior of irrigated rice cultivars in the supply of exudates to the flooded soil for methanogenesis and subsequent CH$_4$ emission.

The present studies highlighted the importance of quantification and further our knowledge on the exuded carbon ratios of the roots of different rice cultivars with respect to the physiological aspects and future possible CH$_4$ emissions by these cultivars. A cultivar may have a good photosynthetic performance that will reflect on its productivity. However, part of that carbon may be translocated to the roots and become available to methanogenic bacteria from the soil. As a result, the most productive cultivar may also be the one that emits more CH$_4$ gas into the atmosphere. Finally, this study elucidated one of the impacts of rice cultivation on the environment and broaden our knowledge of the subject.

**CONCLUSION**

Irrigated rice cultivars show differences between each other in terms of carbon exudation, shoot dry matter, and physiological variables linked to photosynthesis. Early cultivars ‘IRGA 421’, ‘Koshihikari’, and ‘IRGA 430’ were more inefficient in variables physiological parameters reflected in reduced values of exudation and dry matter of the aerial part whereas the cultivars of medium-cycle ‘BRS Taim’ and ‘IRGA 428’ were more efficient with influence in the rate of exudation and dry matter of the aerial part.

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**Table 2** - Phenotypic (rf) and genotypic (rg) correlations between six physiological variables (soil experiment) at 78 days after transplantation (1st reading) and EXRToc exudate of the roots in the full flowering stage (experiment in nutrient solution), evaluated in ten cultivars of irrigated rice under greenhouse conditions.

| Variables | R  | Gs  | Ci  | E   | WUE | A/Ci | EXRtoc |
|-----------|----|-----|-----|-----|-----|------|--------|
| A         | rf | 0.53| -0.61| 0.88""| 0.46| 0.98""| 0.53 |
|           | rg | 0.54| -0.60| 0.90""| 0.46| 0.98""| 0.55 |
| gs        | rf | 0.27| 0.85""| -0.48| 0.40| 0.87""|      |
|           | rg | 0.27| 0.84""| -0.46| 0.42| 0.88""|      |
| ci        | rf | -0.19| -0.96""| -0.72""| 0.28|     |       |
|           | rg | -0.22| -0.96""| 0.71""| 0.28|     |       |
| E         | rf | 0.01| 0.80""| 0.79""|      |      |       |
|           | rg | 0.05| 0.83""| 0.80""|      |      |       |
| WUE       | rf | 0.59|      | -0.41|      |      |       |
|           | rg | 0.59|      | -0.40|      |      |       |
| A/ci      | rf | 0.41|      |      |      |      |       |
|           | rg | 0.43|      |      |      |      |       |

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**Table 2** (continued) - At 93 days after transplantation (2nd reading)

| Variables | R  | Gs  | Ci  | E   | WUE | A/Ci | EXRtoc |
|-----------|----|-----|-----|-----|-----|------|--------|
| A         | rf | 0.63"| -0.97""| 0.83""| 0.83""| 0.99""| 0.68 |
|           | rg | 0.62| -0.97""| 0.83""| 0.83""| 0.99""| 0.68 |
| gs        | rf | -0.47| 0.90""| 0.21| 0.62| 0.38 |       |
|           | rg | -0.48| 0.91""| 0.20| 0.61| 0.38 |       |
| ci        | rf | -0.75"| -0.86""| -0.97""| -0.71"|     |       |
|           | rg | -0.75"| -0.86"| -0.98"| -0.71"|     |       |
| E         | rf | 0.41| 0.83""| 0.67"|      |      |       |
|           | rg | 0.42| 0.84""| 0.67"|      |      |       |
| WUE       | rf | 0.83"|      | 0.41|      |      |       |
|           | rg | 0.83"|      | 0.41|      |      |       |
| A/ci      | rf | 0.70"|      |      |      |      |       |
|           | rg | 0.70"|      |      |      |      |       |

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A = net assimilation rate of CO$_2$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$), Gs = stomatal conductance (mol H$_2$O m$^{-2}$s$^{-1}$), Ci = concentration of intercellular CO$_2$ (μmol CO$_2$ mol$^{-1}$), E = transpiration rate (mmol H$_2$O m$^{-2}$ s$^{-1}$), WUE = water use efficiency (mol CO$_2$ mol H$_2$O$^{-1}$), A/Ci = instantaneous carboxylation efficiency by Rubisco, EXRtoc = total organic carbon exuded from roots (mg C plant$^{-1}$ day$^{-1}$). "", "", "", and "" = significant at 1 and 5% probability by the t-test. "" and "" = significant at 1 and 5% by the Bootstrap method with 5,000 simulations.

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A = net assimilation rate of CO$_2$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$), Gs = stomatal conductance (mol H$_2$O m$^{-2}$s$^{-1}$), Ci = concentration of intercellular CO$_2$ (μmol CO$_2$ mol$^{-1}$), E = transpiration rate (mmol H$_2$O m$^{-2}$ s$^{-1}$), WUE = water use efficiency (mol CO$_2$ mol H$_2$O$^{-1}$), A/Ci = instantaneous carboxylation efficiency by Rubisco, EXRtoc = total organic carbon exuded from roots (mg C plant$^{-1}$ day$^{-1}$). "", "", "", "", and "" = significant at 1 and 5% probability by the t-test. "" and "" = significant at 1 and 5% by the Bootstrap method with 5,000 simulations.
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DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest which could constitute an impediment to the publication of this article.

AUTHORS’ CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

AULAKH, M. S. et al. Impact of root exudates of different cultivars and plant development stages of rice (Oryza sativa L.) on methane production in a paddy soil. Plant and Soil, v. 230, p.77-86, 2001. Available from: <https://link.springer.com/article/10.1023/A:1004817212321>. Accessed: Apr. 23, 2016. doi: 10.1023/A:1004817212321.

BHATTACHARYYA, P. et al. Influence of elevated carbon dioxide and temperature on belowground carbon allocation and enzyme activities in tropical flooded soil planted with rice. Environmental Monitoring and Assessment, v. 185, p. 8659-8671, 2013. Available from: <https://link.springer.com/article/10.1007/s10661-013-3202-7>. Accessed: Mar. 15, 2017. doi: 10.1007/s10661-013-3202-7.

COUNCE, P.A. et al. A uniform, objective, and adaptive system for expressing rice development. Crop Science, v. 40, p. 436-443, 2000. Available from: <https://pdfs.semanticscholar.org/b054/14c3be8fde57e1260f5386395a62e9c993e.pdf>. Accessed: Jun. 16, 2017. doi: 10.2135/cropsci2000.402436x.

CRUZ, C. D. Programa Genes: Análise multivariada e simulações. Viçosa: Universidade Federal de Viçosa (UFV), 2006. Available from: <http://arquivo.ufv.br/dhg/genes/gdow1.htm>. Accessed: Apr. 14, 2016.

EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos. 3º ed. rev. ampl. Brasilia, 2013. 353 p.

FURLANI, A.M.C.; FURLANI, P.R. Composição e pH de soluções nutritivas para estudos fisiológicos e seleção de plantas em condições nutricionais adversas. Campinas: Instituto Agronômico, 1988. 34p. (Technical bulletin, 121).

GARCIA-ORENSES, F. et al. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. Soil & Tillage Research, v. 109, p. 110-115, 2010. Available from: <http://www.sciencedirect.com/science/article/pii/S0167198710000838>. Accessed: Mar. 05, 2017. doi: 10.1016/j.still.2010.05.005.

GU, J. et al. Physiological basis of genetic variation in leaf photosynthesis among rice (Oryza sativa L.) introgression lines under drought and well-watered conditions. Journal of Experimental Botany, v. 63, p. 5137-5153, 2012. Available from: <https://academic.oup.com/jxb/article/63/14/5137/536113>. Accessed: Mar 18, 2018. doi: 10.1093/jxb/erq170.

ITOH, M. et al. Mitigation of methane emissions from paddy fields by prolonging midseason drainage. Agriculture, Ecosystems and Environment, v. 141, p. 359-372, 2011. Available from: <https://www.sciencedirect.com/science/article/pii/S0167880911001034>. Accessed: Mar. 30, 2018. doi: 10.1016/j.agee.2011.03.019.

KONDO, M. et al. Anatomy of nodal roots in tropical upland and lowland rice varieties. Plant Production Science, v. 3, p. 437-445, 2000. Available from: <https://www.tandfonline.com/doi/abs/10.1626/pps.3.437>. Accessed: Mar. 23, 2018. doi: 10.1626/pps.3.437.

LORENCONI, R. CO assimilation of modern and traditional rice genotypes subjected to water stress. Brazilian journal of agriculture-Revista de Agricultura, v. 89, p. 127-140, 2014. Available from: <http://www.afeaq.org.br/ojs/index.php/revistadecultura/article/view/60>. Accessed: Feb. 18, 2018.

PEARCY, R.W.; PFITSCH, W.A. Influence of sunflecks on the δ 13 C of Adenanourl bicolor plants occurring in contrasting forest understory microsites. Oecologia, v. 86, p. 457-462, 1991. Available from: <https://link.springer.com/article/10.1007/BF00318310>. Accessed: Jun. 18, 2018. doi: 10.1007/BF00318310.

PUPATTO, J.G.C. Trocas gasosas e eficiência de uso da água da cultura do arroz irrigado por aspersão em função da aplicação de silício. 2003. 166 f. Tese (Doutorado em Agronomia) Curso de Pós-graduação em Agricultura, Universidade Estadual Paulista (UNESP). Available from: <https://repositorio.unesp.br/handle/11449/99995>. Accessed: Dec. 03, 2017.

SHIMAKURA, S.E.; RIBEIRO JÚNIOR, P.J. Estatística descritiva: interpretação do coeficiente de correlação. Departamento de Estatística da Universidade Federal do Paraná (UFPR), 2012. Available from: <http://leg.ufpr.br/~ce003/ce003/nodel8html>. Accessed: Apr.16, 2017.

SILVA, E.G. et al. Gas exchange and chlorophyll fluorescence of eggplant grown under different irrigation depths. Revista Brasileira de Engenharia Agrícola eAmbiental, v. 19, p. 946-952, 2015. Available from: <http://www.agriambi.com.br/revista/v19n10/v19n10a06.pdf>. Accessed: Mar. 25, 2018. doi: 10.1590/1807-1929/agriambi.v19n10p946-952.

SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO (SOSBAI). Reunião Técnica da Cultura do Arroz Irrigado Arroz irrigado (2016): recomendações técnicas da pesquisa para o Sul do Brasil. Pelotas, RS: SOSBAI, 2016. 200 p.

SOUZA, C.A.V. Mudanças foto-oxidativas in plantas de arroz com reduzida atividade do oxidase do glicolato submetidas ao estresse hídrico combinado ao excesso de luz e calor. 175 f. Dissertação (Mestrado em Produção Vegetal) Curso de Pós-graduação em Produção vegetal, Universidade Federal Rural de
SU, J et al. Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice. *Nature*, v. 523, p. 602-620, 2015. Available from: <https://www.nature.com/articles/nature14673>. Accessed: May 15, 2017. doi: 10.1038/nature14673.

TAIZ, L. et al. *Fisiologia e desenvolvimento vegetal*. 6. Ed. Porto Alegre: Artmed, 2017. 888 p.

YIQI, L.; ZHOU, X. *Soil respiration and the environment*. San Diego: Elsevier, 2010. 307p.

YOSHIDA, S. et al. *Laboratory Manual for Physiological Studies of Rice*. Los Baños: IRRI, 1976. 83p.