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Correlation between carbon isotopic composition and morphological, micromorphological, anatomical, and physiological traits in rice

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Abstract – The objective of this work was to verify the correlations between carbon isotopic composition and traits of superior rice genotypes. Twenty genotypes were analyzed for morphology, micromorphology, anatomy, physiological performance, and carbon fingerprint. The plots consisted of 500 L plastic boxes sowed with rice, to allow of a plant density of 300 plants per square meter. Plant anatomy and physiological performance were evaluated using a microscope and an infrared gas analyzer, respectively. There is a correlation between rice water use efficiency (r = 0.45) and carboxylation efficiency (r = 0.39).

Index terms: *Oryza sativa*, carbon fingerprint, gas exchange, plant breeding.

Correlação entre composição isotópica de carbono e traços morfológicos, micromorfológicos, anatômicos e fisiológicos de arroz

Resumo – O objetivo deste trabalho foi verificar as correlações entre a composição isotópica de carbono e as características de genótipos superiores de arroz. Vinte genótipos foram analisados quanto à morfologia, à micromorfologia, à anatomia, ao desempenho fisiológico e à composição isotópica de carbono. As parcelas consistiram em caixas de plástico de 500 L com arroz semeado, para permitir a densidade de 300 plantas por metro quadrado. A anatomia e o desempenho fisiológico das plantas foram avaliados em microscópio e com analisador de gases por infravermelho, respectivamente. Há correlação entre a eficiência do uso da água do arroz (r = 0,45) e a eficiência da carboxilação (r= 0,39).

Termos para indexação: *Oryza sativa*, composição isotópica de carbono, trocas gasosas, melhoramento de plantas.

Carbon can be found in the atmosphere in three forms: ^{12}C, ^{13}C, and ^{14}C, and being ^{12}C (~98.9%) and ^{13}C (~1.1%) are stable isotopes (Gromov et al., 2017). The ^{14}C radioisotope is much rare (< 0.0001%). The isotopic abundance of ^{12}C and ^{13}C is unlikely to change in atmosphere in the short term, as they are stable (February & Stock, 1999).

Plant physiologists found a differentiation on the C isotope incorporation into plant materials as function of the first enzyme on the

photosynthesis pathway (Ivlev, 2001). This pathway is related to micromorphological, anatomical, and biochemical traits. The C isotope incorporation can be used as a tool to determine whether a given plant with unknown carbon metabolism is C_3, C_4 or C_3 - C_4 intermediate (Menezes Neto & Guerra, 2019).

Although there is a general knowledge of the involvement of micromorphological, anatomical, and biochemical plant traits in carbon fingerprint; these relations are yet to be further established and understood. In applied sciences, it would be noteworthy to establish a clear correlation between carbon fingerprint and overall plant performance. It would summarize the behavior of the morphophysiological pathway, from CO_2 entry into leaves until its release through respiration. Other applications may be widely open, if a consistent correlation is established between carbon fingerprint and morphophysiological traits of crop plants. Breeding programs could benefit from this knowledge for most accurate plant selection and development of superior cultivars. Despite their importance for performance assessment of superior plants, these traits are not easy or practical to be used in a breeding program. The number of genotypes to be profiled may be too high in the first steps of the process. The verification of correlations among these traits to a faster-assessed variable would benefit rice breeding programs worldwide.

The objective of this work was to verify the correlations between carbon fingerprints of superior rice varieties and their morphological, micromorphological, anatomical, and physiological traits.

A randomized block experimental design with four replicates was carried out at Terras Baixas experimental station, of Embrapa Clima Temperado, in the municipality of Capão do Leão, in the state of Rio Grande do Sul, Brazil, during the 2019/2020 cropping season. Each plot was a rectangular 500 L fiberglass water tank, equipped with water inlets and outlets, and a valve-control, for the precise irrigation and drainage as needed. The tanks were filled from bottom to top, as follows: 5 cm layer of crushed stone; 5 cm layer of coarse sand; and 50 cm layer of Planosol, which was previously corrected, fertilized for rice, as well as subject to topdressing fertilization, according to the recommendations by the Instituto Rio Grandense de Arroz (IRGA) (Boletim..., 2020).

Treatments consisted of 20 superior rice genotypes (lineages and commercially available cultivars). Each tank was sowed with all 20 genotypes, in 1 m long rows, spaced at 0.175 m from each other. Each line corresponded to one genotype. Ten days after the emergence, plant density was adjusted to 300 plants per square meter. The cycle length of the genotypes ranged from 115 to 135 days from emergence to harvest. Planting was performed on 09/23/ 2019, and emergence occurred on 10/2/2019.

Irrigation was managed in an alternate wetting and drying system (AWD) (Sriphirom et al., 2019). From rice sowing to its tillering starting, all plots were irrigated daily to keep soil moist. At tillering, a 7 cm water layer was applied. Plots were not irrigated again until soil water tension reached 20 kPa. At this water tension, a new 7 cm water layer was applied, and a new cycle of natural drainage initiated (Pinto et al., 2016). Cycles were repeated until ripening started. Two sensors per plot were used to monitor water tension in the soil, a Watermark Soil Moisture Sensor (Irrometer Company, Inc., Riverside, CA, USA) wired to the IRR 900M Watermark Monitor electronic data logger (Irrometer Company, Inc., Riverside, CA, USA). Data reading was carried out twice a day.

The plant morphological analysis was performed 22 days after plant emergence (DAE). For each plot, two rice plants were collected per genotype (4 replicates × 2 plants = 8 plants per genotype), deposited into a glass with water and immediately taken to the lab. The sample size was 40 plants per plot. In the lab, the second leaf (first expanded leaf) and root lengths were measured. The number of tillers and roots were counted (Table 1). Leaf length was measured with a graduated ruler from leaf collar to its tip. Root length was measured from seed insertion to the longest root tip. After the assessments, the second leaf (first expanded leaf) was removed for the micromorphological analysis.

The middle section of the adaxial (upper) face of the second leaf was observed and photographed using the Nikon e200 optical microscope (Nikon Instruments Inc., Melville, NY, USA). Photography scales were previously set using a microscope calibration ruler. Images were saved and lately processed using the software ImageJ v.1.53c. Nervure density, internervure distance, stomatal density and opening width were measured (Table 1). Stomata were counted in an area

corresponding to 25% of the captured image, as this was the area where the microscope had better focus.

Leaf samples were collected for anatomical assessments at 110 DAE. A 10 cm section was cut from the middle third of the second leaf of rice plants. Two samples were obtained per replicate and lineage. These samples were put into acrylic flasks and fully covered with alcohol 70%, sealed and stored at 5±1ºC in a refrigerator. The leaf samples were cut using a good quality razor blade, in accordance with standard laboratory routines, and photographed using the microscope. Percentages of aerenchyma in the central nervure of rice leaf, xylem and phloem vessels, and sclerenchyma area were also measured in the photographs using ImageJ v.1.53c (Schneider et al., 2012), as shown in Table 1.

The physiological performance of genotypes was evaluated at 51 and 57 DAE in two blocks of the experiment (two samples per genotype) each day, using the GFS-3000 infrared gas analyzer (Heinz Walz GmbH, Effeltrich, Germany). In all evaluations, the following environmental parameters were set up through the infrared gas analyzer: 750 µmol s^{-1} flow; seven impeller steps; 372 µmol mol^{-1} ± 3.75% environmental CO_2 concentration; PARTop light mode; abs H_2O mode; 1,000 µmol m^{-2} s^{-1} light; Tcuv temp mode. Reference air was captured at 3.5 m above the soil level. The following parameters were assessed: net photosynthesis; transpiration rate; vapor pressure gradient; stomatal conductance; internal CO_2; water use efficiency; intrinsic water use efficiency; carboxylate efficiency; and temperature gradient between leaf and cuvette (Table 1). Genotypes were also read in random order each day, to compensate for time change as day passed.

Table 1. Spearman's rank correlation between carbon fingerprint ($\delta^{13}C$) and the evaluated variables ($\alpha = 0.05$).

Variable	Carbon fingerprint ($\delta^{13}C$)	
	Spearman	Sig.
2nd leaf length	0.06	0.798*
Root length	0.10	0.672
Number of roots	0.09	0.710*
Number of tillers	-0.34	0.141*
Nervure density	0.23	0.334
Internervure distance	0.22	0.361
Stomatal density	0.02	0.921*
Stomatal width	-0.08	0.740*
Xylem vessel area	0.04	0.860*
Phloem vessel bundle area	-0.04	0.871
Sclerenchyma in vessel bundle	0.01	0.967
Aerenchyma (%) in central nervure	0.17	0.476*
Transpiration rate	0.09	0.695
Vapor pressure gradient	-0.12	0.617
Photosynthesis rate	0.17	0.280
Internal CO_2	-0.15	0.538
Water use efficiency	0.45	0.003
Stomatal conductance	0.04	0.871
Intrinsic water use efficiency	0.16	0.509
Carboxylation efficiency	0.39	0.092*
Temperature gradient	0.09	0.722*

*Ties do not allow of the exact p determination by the Spearman's test.
Source: Embrapa Clima Temperado, Pelotas, RS, Brazil, 2019-2020.

The isotopic carbon composition (carbon fingerprint) was determined using an isotopic mass spectrometer at the Centro de Isótopos Estáveis of the Universidade Estadual Paulista (Unesp), Brazil. Carbon isotope percentage in the leaves was determined using the Delta S isotope ratio mass spectrometer (IRMS) (Finnigan Mat, Bremen, Germany). Leaf samples were dried in an oven at 50ºC for 48 hours and later homogenized in a cryogenic mill 2010 Geno/Grinder (SPEX SamplePrep, Metuchen, NJ, USA), using liquid nitrogen at -196ºC. An aliquot from 50 to 7 µg of each sample was weighed in a tin capsule, using a 1 µg resolution scale on the XP6 micro balance (Mettler-Toledo, Greifensee, Switzerland). The sample homogenization increases the accuracy of small samples. Capsules were analyzed in a continuous flow isotope ratio spectrometry system (CF-IRMS) using the Delta V IRMS (Thermo Fisher Scientific, Waltham, MA, USA) coupled to the elemental analyzer Flash 2000 (Thermo Scientific, Waltham, MA, USA), using the ConFlo IV gas interface (Thermo Scientific, Waltham, MA, USA). The CF-IRMS determined the carbon isotopic ratio ($R\ ^{13}C/^{12}C$), which was was expressed in milli Urey (mUr) (Brand & Coplen, 2012). The isotopic composition ($\delta^{13}C$) was measured as the relative difference between isotopic ratio ($R\ ^{13}C/^{12}C$) and the VPDB standard (NBS-22) normalized in accordance with the VPDB standard (Coplen, 2011). The standard uncertainty of the CF-IRMS is ±0.15 mUr. The $\delta^{13}C$ values were calculated using the following equation:

$$\delta^{13}C = \frac{\left(R^{13}C/^{12}C\right)\text{sample}}{\left(R^{13}C/^{12}C\right)\text{VPDB}} - 1$$

where: $\delta^{13}C$ is the carbon isotopic composition (carbon

fingerprint); R $^{13}C/^{12}C = {}^{13}C/^{12}C$ is the carbon ratio of the sample or the VPDB standard.

The statistical analysis was carried out using the R v. 4.1.2 (R Core Team, 2021). Data sets were tested for homoscedasticity using the Levene's test. The assumption was not met for any of the variables. Nonparametric correlation was used. A two-tailed Spearman's rank correlation was used to verify the relationship between carbon isotopic composition ($\delta^{13}C$) and all measured variables (Table 1). All analyses were considered at the 5% threshold level. Sixteen variables showed a very weak positive correlation ($r < 0.20$) with isotopic composition that was not significant. The variables were the second leaf length, root length, root number, stoma density, stoma width, xylem area, phloem area, sclerenchyma, aerenchyma, transpiration rate, vapor pressure, photosynthesis, internal CO_2, stoma conductance, intrinsic water use efficiency (WUE), and temperature gradient. Three variables — tiller number, nervure density and internervure distance — showed a weak positive correlation ($0.20 \leq r < 0.40$) with isotopic composition, but they were not significant. Only the variables WUE and carboxylation efficiency showed a significant correlation. WUE showed a moderate positive correlation ($0.40 \leq r < 0.60$). Carboxylation efficiency showed a weak positive correlation.

The correlation between WUE and carbon fingerprint was 45% ($p=0.003$). The correlation between carboxylation efficiency and carbon fingerprint was 39% ($p=0.092$). Many abiotic factors can influence these relationships. The major ones are soil moisture, air humidity, stoma density and opening width, wind speed, radiation intensity, and CO_2 metabolism type (Haworth et al., 2016). In tropical environments, water is usually the scarcest resources and the most accountable for grain yield losses. This is especially true for rice (Lauteri et al., 2014).

In rice cropping systems, most of the advances in WUE were accomplished by reducing water losses, when storing, pumping or distributing water. In the past 15 years, the use of such advances increased the WUE from 0.50 kg m^{-3} to 0.94 kg m^{-3}. However, there is an important contribution of rice cultivars with superior physiological WUE (Concenço et al., 2020). WUE reached 1.3 kg m^{-3}, when superior varieties were used (Tortelli et al., 2019).

The carboxylation efficiency is estimated based on the photosynthesis rate as a function of the available CO_2 into the leaf mesophyll (Rho et al., 2011). The presence of ties in the data sets did not allow of the exact p determination by using the Spearman's rank correlation (Table 1).

Rice WUE and carbon fingerprint show a moderate positive correlation of 45%. Carboxylation efficiency and carbon fingerprint ($\delta^{13}C$) show a weak correlation of 39%. Breeding rice genotypes based in their carbon fingerprint ($\delta^{13}C$) will also indirectly select for increased efficiency of both water use and carboxylation.

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References

BOLETIM DE RESULTADOS DA LAVOURA - SAFRA 2019/2020. Porto Alegre: Irga, 2020. Available at: <https://irga.rs.gov.br/upload/arquivos/202008/19144808-boletim-de-resultados-da-lavoura-safra-2019-2020-irga.pdf>. Accessed on: Jan. 17 2021.

BRAND, W.A.; COPLEN, T.B. Stable isotope deltas: tiny, yet robust signatures in nature. **Isotopes in Environmental and Health Studies**, v.48, p.393-409, 2012. DOI: https://doi.org/10.1080/10256016.2012.666977.

CONCENÇO, G.; PARFITT, J.M.B.; SINNEMANN, C.S.; CAMPOS, A.D.S.; VEIGA, A.B.; BERGMANN, H.M.; MELO, T.S.; SILVA, L.B.X. Semeadura direta de arroz em resteva de soja cultivada no sistema sulco-camalhão. **Brazilian Journal of Development**, v.6, p.13221-13231, 2020. DOI: https://doi.org/10.34117/bjdv6n3-258.

COPLEN, T.B. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. **Rapid Communications in Mass Spectrometry**, v.25, p.2538-2560, 2011. DOI: https://doi.org/10.1002/rcm.5129.

FEBRUARY, E.C.; STOCK, W.D. Declining trend in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide from tree rings of South African *Widdringtonia cedarbergensis*. **Quaternary Research**, v.52, p.229-236, 1999. DOI: https://doi.org/10.1006/qres.1999.2057.

GROMOV, S.; BRENNINKMEIJER, C.A.M.; JÖCKEL, P. Uncertainties of fluxes and $^{13}C/^{12}C$ ratios of atmospheric reactive-gas emissions. **Atmospheric Chemistry and Physics**, v.17, p.8525-8552, 2017. DOI: https://doi.org/10.5194/acp-17-8525-2017.

HAWORTH, M.; KILLI, D.; MATERASSI, A.; RASCHI, A.; CENTRITTO, M. Impaired stomatal control is associated with reduced photosynthetic physiology in crop species grown at elevated [CO_2]. **Frontiers in Plant Science**, v.7, art.1568, 2016. DOI: https://doi.org/10.3389/fpls.2016.01568.

IVLEV, A.A. Carbon isotope effects ($^{13}C/^{12}C$) in biological systems. **Separation Science and Technology**, v.36, p.1819-1914, 2001. DOI: https://doi.org/10.1081/SS-100104762.

LAUTERI, M.; HAWORTH, M.; SERRAJ, R.; MONTEVERDI, M.C.; CENTRITTO, M. Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. **PLoS ONE**, v.9, e109054, 2014. DOI: https://doi.org/10.1371/journal.pone.0109054.

MENEZES NETO, M.A.; GUERRA, M.P. A new method for determination of the photosynthetic pathway in grasses. **Photosynthesis Research**, v.142, p.51-56, 2019. DOI: https://doi.org/10.1007/s11120-019-00646-5.

PINTO, M.A.B.; PARFITT, J.M.B.; TIMM, L.C.; FARIA, L.C.; SCIVITTARO, W.B. Produtividade de arroz irrigado por aspersão em terras baixas em função da disponibilidade de água e de atributos do solo. **Pesquisa Agropecuária Brasileira**, v.51, p.1584-1593, 2016. DOI: https://doi.org/10.1590/S0100-204X2016000900058.

R CORE TEAM. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2021. Available at: <http://www.R-project.org/>. Accessed on: Jan. 10 2021.

RHO, H.; YU, D.J.; KIM, S.J.; CHUN, C.; LEE, H.J. Estimation of carboxylation efficiency from net CO_2 assimilation rate as a function of chloroplastic CO_2 concentration in strawberry (*Fragaria* × *ananassa* cv. Maehyang) leaves. **Horticulture, Environment, and Biotechnology**, v.52, p.547-552, 2011. DOI: https://doi.org/10.1007/s13580-011-0035-6.

SCHNEIDER, C.A.; RASBAND, W.S., ELICEIRI, K.W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, v.9, p.671-675, 2012. DOI: https://doi.org/10.1038/nmeth.2089.

SRIPHIROM, P.; CHIDTHAISONG, A.; TOWPRAYOON, S. Effect of alternate wetting and drying water management on rice cultivation with low emissions and low water used during wet and dry season. **Journal of Cleaner Production**, v.223, p.980-988, 2019. DOI: https://doi.org/10.1016/j.jclepro.2019.03.212.

TORTELLI, G. de S.; HERNANDES, G.; PARFITT, J.M.B.; CONCENÇO, G.; CAMPOS, A.D.S. de; AIRES, T.A.; ANDRES, A. Arroz irrigado por aspersão no Rio Grande do Sul: situação na safra 17/18. In: CONGRESSO BRASILEIRO DE ARROZ IRRIGADO, 11., 2019, Balneário Camboriú. **Inovação e desenvolvimento na orizicultura**: anais eletrônicos. Itajaí: Epagri/Sosbai, 2019.