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SCIENTIFIC COMMUNICATION

CARBON ISOTOPE DISCRIMINATION IN MANGO LEAVES UNDER SALT STRESS[1]

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ABSTRACT – The aim of this study was to evaluate the response of mango plants (*Mangifera indica* L) 'Tommy Atkins' cultivar to different NaCl concentrations. In this experiment, plants were grown in pots with washed sand and nutrient solution with 0, 10, 20 and 30 mM NaCl for 120 days. After this period, young leaves from the last growth spurt were harvested in a randomized block design, dried at 65 °C for 72 h and ground in a ball mill. Samples were dried again in an oven at 60 °C for 24 h and then the relative abundance of ^{13}C and ^{12}C isotopes was determined using mass spectrometer Sercon 20-20 isotope ratio model. Data were used to determine the carbon isotopic discrimination (Δ). It was concluded that 'Tommy Atkins' mango trees submitted to 30 mmol L^{-1} NaCl discriminated less ^{13}C, which may be an indication of salt stress in these plants.
Index terms: *Mangifera indica*, salinity, isotopes.

DISCRIMINAÇÃO ISOTÓPICA DE CARBONO EM FOLHAS DE MANGUEIRAS SUBMETIDAS A ESTRESSE SALINO

RESUMO – Com o objetivo de estudar a resposta de plantas de mangueira (*Mangifera indica* L.) 'Tommy Atkins' a diferentes concentrações de NaCl, um experimento foi conduzido, sendo as plantas cultivadas em vasos com areia lavada e solução nutritiva, com 0; 10; 20 e 30 mM de NaCl por 120 dias. Após esse período, folhas jovens, do último surto de crescimento, foram colhidas num delineamento em blocos casualisados, secas em estufa a 65 °C, por 72 h, e moídas em moinho de bola. As amostras foram então novamente submetidas à secagem em estufa a 60 °C, por 24 h, e após foi então determinada a abundância relativa dos isótopos ^{13}C e ^{12}C, utilizando-se de espectrômetro de massa de razão isotópica modelo Sercon 20-20. Com esses dados, determinou-se a discriminação isotópica do carbono (Δ). Concluiu-se que mangueiras 'Tommy Atkins' submetidas a 30 mmol L^{-1} de NaCl discriminaram menos o ^{13}C.
Termos para indexação: *Mangifera indica*, salinidade, isótopos.

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Salt stress inhibits plant growth by osmotic effect, restricting water availability due to the toxic effect of specific ions such as Na^+ and Cl^- (FLOWERS; FLOWERS, 2005) and nutritional disorder may also occur (AL-YASSIN, 2004), which can lead to morphological changes that are more marked than the osmotic effect (EPSTEIN; BLOOM, 2006). Studies on the effects of Na + on tree species are still very limited. The literature reports that lesions caused by Na + at concentrations below 5 mmol L^{-1} in the soil solution is common in avocado (*Persea americana* Mill.), citrus (*Citrus* spp.) and stone fruit trees (*Prunus* spp.) (MAAS, 1990). After absorption by roots, Na + is translocated to the aerial part of the plant, causing the burning of leaves. Most cultivated fruit species are classified as salt-sensitive (RHOADES; LOVEDAY, 1990).

Symptoms of lesions due to excessive salinity may take a long time to appear after exposure to salinity as some more sensitive fruit trees may accumulate sodium or chloride or both over time, even if grown on soils classified as with low saline or low sodium contents. Salinity tolerance is therefore variable among species and within a single species, and may be specific at a given stage of development, and in the same genotype, it may be more tolerant at one stage and more sensitive at another (BERNSTEIN, 1975; FOOLAD et al., 1998, ORCUTT; NILSEN, 2000).

In the last three decades, there has been a growing use of stable isotopes in the most different fields of science. Isotopic analyses are currently considered as an important tool for physiologists, ecologists and other researchers who study the cycles of elements (PEREIRA, 2007), in the analysis of the effects of stress on plants (MACHADO et al., 1992), in the analysis of flows and metabolic routes (RATCLIFFE and SHACHAR-HIL, 2006) and, to a large extent, in the study of soil organic matter (FERNANDES et al., 2007).

There are two carbon isotopes (C) that are stable and are found in nature in defined proportions, which remain relatively stable in any organic residue: ^{12}C is the lightest and presents the largest proportion in relation to total C in nature (98.89%), and ^{13}C accounts for only 1.11% of total C (ALVES et al., 2005). During the process of photosynthetic CO_2 absorption, all plants perform a discrimination against heavier molecules of this gas, preferring those containing ^{12}C, rather than those with ^{13}C, causing them to have a lower content of ^{13}C than atmospheric CO_2 (MARTINEZ et al., 2007).

Isotopic composition analyses of C are made by measuring the ^{13}C / ^{12}C ratio of samples against an international standard, and the result is expressed in terms of $\delta^{13}C$ difference from the standard. The international standard for carbon is a limestone found in a geological formation called Pee Dee, North Carolina, USA, whose abbreviation is PDB (Pee Dee Belemnite), and has ^{13}C / ^{12}C (R) molar ratio of 0.01124. Deviations from the standard are known as δ units (lower case delta Greek letter). As values are very small, it was agreed to express them in parts per thousand (‰). The result is expressed in terms of the isotopic composition difference ($\delta^{13}CPDB$) in relation to PDB, usually generating a negative value, since the ^{13}C / ^{12}C molar ratio of plants is lower than PDB (O'LEARY, 1981; MACHADO et al., 2011). According to SMITH and EPSTEIN (1970), $\delta^{13}C$ PDB values of CO_2 in the atmosphere are around -8 ‰, and in plants are from -9 to -34 ‰.

In plants, which can be divided into three major photosynthetic groups, C3, C4 and CAM, where each group has a specific isotopic discrimination standard (MACHADO et al., 1992), much of the natural isotopic variation results from isotopic discrimination during photosynthesis, which is altered in stress situations.

The $\delta^{13}C$ PDB of these different photosynthetic groups is generally a result of the biochemical properties of enzymes in the primary CO_2 fixation and CO_2 diffusion limitations within leaves (FARQUHAR et al., 1989). The high salinity of irrigation water, for example has a detrimental effect on the stomatal opening process of plants by increasing the resistance to CO_2 diffusion (FLEXAS et al., 2008). Similar to salt stress, according to PATE (2001), under conditions of water stress, C_3 cycle plants tend to discriminate less ^{13}C, which seems to be a tool for the selection of varieties more suitable for more arid climates.

Thus, the knowledge of the tolerance level of species to salt stress is very important so that alternative management techniques can be used with the purpose of mitigating the detrimental effects to the culture, when cultivated in soils with high salt levels.

The aim of this study was to verify the occurrence of salt stress in 'Tommy Atkins' mango plants submitted to different salinity levels through the carbon isotope discrimination technique (Δ).

Mango trees (*Mangifera indica* L.), Tommy Atkins cultivar, grafted on Imbú variety, were planted in pots with washed sand and nutrient solution of Hoagland and Arnon (HOAGLAND; ARNON, 1950) and submitted to NaCl concentrations of 0, 10, 20 and 30 mmol L^{-1} on 09/23/2011. The experimental design was a randomized block design. On January

23, 2012, after 120 days of plant permanence in saline solutions, young leaves were collected from the last growth spurt, and two plants were sampled per treatment with five replicates.

After collection, leaves were dried in an oven with forced air ventilation at 65°C for 72 hours. Then, they were pre-ground in a crucible, placed in 3 ml eppendorf tubes and milled in a ball mill at the Laboratory of Molecular Physiology of Plants, Department of Plant Biology, UFV. Samples were again submitted to oven drying at 60°C for 24 h and sent to the Laboratory of Stable Isotopes, Department of Soils, UFV, where the relative abundance of ^{13}C and ^{12}C isotopes was determined using mass spectrometer of isotope ratio model Sercon 20-20. Results were used to determine the carbon isotope discrimination (Δ). The carbon isotopic composition was expressed in relation to the Pee Dee Belemnite standard (FARQUHAR et al., 1989).

Table 1 shows that plants submitted to treatment with 30 mM L^{-1} of NaCl discriminated less ^{13}C than those submitted to the other treatments.

According to ALVES et al. (2005), C_3 cycle plants, such as mango trees, which are not being submitted to stress conditions, discriminate more intensely ^{13}C and therefore present variations in the average isotopic abundance of ^{13}C of -27 ‰, on average. According to LARCHER (2006), the occurrence of ^{13}C in the vegetable mass is usually low, because during carboxylation, ^{12}C is favored by enzymes. Rubisco favors more strongly ^{12}C, discriminating more ^{13}C. As a consequence, C_3 terrestrial plants have low $\delta^{13}C$ values (-23 to -36 ‰, on average, -27 ‰), compared to C_4 plants.

It was concluded that 'Tommy Atkins' mango trees submitted to 30 mmol L^{-1} of NaCl discriminate less ^{13}C.

TABLE1- Mean isotopic values in δ ‰ ^{13}C for mango trees submitted to different NaCl concentrations.

Samples[1]	δ ‰ ^{13}C (μ)
0 mmol L^{-1} of NaCl	-28.12a
10 mmol L^{-1} of NaCl	-28.37a
20 mmol L^{-1} of NaCl	-28.65a
30 mmol L^{-1} of NaCl	-22.61b

[1] Means followed by equal letters do not differ significantly from each other by the Tukey test at 5% probability.

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REFERENCES

ALVES, B.J.R.; ZOTARELLI, L.; JANTALIA, C.P.; BODDEY, R.M.; URQUIAGA, S. Emprego de isótopos estáveis para o estudo do carbono e nitrogênio no sistema solo-planta. In: AQUINO, A.M.; ASSIS, R.L. (Org.). **Processos biológicos no sistema solo-planta**: ferramentas para uma agricultura sustentável. Brasília, DF: Embrapa Informação Tecnológica, v.1, p.343-368, 2005.

AL-YASSIN, A. Influence of salinity on citrus: a review paper**. Journal of Central European Agriculture**, Zagreb, v. 5, n. 4, p. 263-272, 2004.

BERNSTEIN, L. Effects of salinity and sodicity on plant growth. **Annual Review of Plant Phytopathology**, Stanford, v.13, p.295-312, 1975.

EPSTEIN, E.; BLOOM, A.J. **Nutrição mineral de plantas**: princípios e perspectivas. 2.ed. Londrina: Planta, 2006. 403p. FARQUHAR, G.D.; EHLERINGER, J.R.; KUBICK, K.T. Carbon isotope discrimination and photosynthesis. **Annual Review of Plant Physiology and Plant Molecular Biology**, Los Angeles, v.40, p.503-537, 1989.

FERNANDES, F.A.; CERRI, C.C.; FERNANDES, A.H.B.M. **^{13}C e a dinâmica do carbono orgânico do solo em pastagem cultivada no Pantanal Sul-Mato-Grossense**. Corumbá: Embrapa Pantanal, 2007. (Boletim de Pesquisa e Desenvolvimento, 74).

FLEXAS, J.; RIBAS-CARBÓ., M.; DIAZ-ESPEJO, A.; GALMÉS, J.; MEDRANO, H. Mesophyll condutance to CO_2: current knowledge and future prospects. **Plant Cell and Environment**, Oxford, n.31, p.602-621, 2008.

FLOWERS, T.J.; FLOWERS, S.A. Why does salinity pose such a difficult problem for plant breeders? **Agricultural Water Management**, New York, v.78, p.15-24, 2005.

FOOLAD, M.R.; CHEN, F.Q.; LIN, G.Y. RFLP mapping of QTLs conferring salt tolerance during germination in an interspecific cross of tomato. **Theoretical and Applied Genetics**, Berlin, v.97, p.1133-1144, 1998.

HOAGLAND, D.; ARNON, D.I. **The water culture method for growing plants without soil.** Berkeley: California Agriculture Experimental Station, 1950. 347 p. (Circular)

LARCHER, W. **Ecofisiologia vegetal**. São Carlos: RiMa, 2006. 550p.

MAAS, E.V. Crop salt tolerance. In: TANJI, K.K. (Ed.). **Agricultural salinity assessment and management**. New York: Americam Society of Civil Engineers, p.262-304, 1990.

MACHADO, D.N.; NOVAIS, R.F.; DILVA, I.R.; LOUREIRO, M.E.; MILAGRES, J.J.; SOARES, E.M.B. Enriquecimento e alocação de ^{13}C em plantas de eucalipto. **Revista Brasileira de Ciência do Solo,** Viçosa, MG, v.35, p.857-866, 2011.

MACHADO, D.N.; NOVAIS, R.F.; SILVA, I.R.; LOUREIRO, M.E.; MILAGRES, J.J.; MARTINELLI, L. A. ^{15}N natural abundance in plants of the Amazon river floodplain and potential atmospheric N_2 fixation. **Oecologia**, Berlin, v. 90, p. 591-596, 1992.

MARTINEZ, J.C.; DUCATTI, C.; SILVA, E.T.; SOUZA, C.R.; MARTINEZ, M.G.; SANTOS, L.C. Uso de Regressão linear em análises isotópicas ($^{13}C/^{12}C$), ($^{15}N/^{14}N$), obtidas por espectrometria de massas, necessária a detecção de adulteração de marcas comerciais de pó de café com produtos de ciclo fotossintético C_4 (milho). In: CONGRESSO NACIONAL DE MATEMÁTICA APLICADA E COMPUTACIONAL, 30., 2007, Florianópolis. **Anais...**

NIELSEN, D. R. (Ed.). **Irrigation of agricultural crops.** Madison: SSSA, 1990. p.1089-1142. (Agronomy Monograph, 30)

O'LEARY, M.H. Carbon isotope fractionation in plants. **Phytochemistry**, Nantes, v.20, p.553-67, 1981.

ORCUTT, D.M.; NILSEN, E.T. **Physiology of plants under stress**. New York: John Willey & Sons, 2000. 225p.

PATE, J.S. Carbon isotope discrimination and plant water-use efficiency: case scenarios for C3 plants. In: UNKOVICH, M.; PATE, J.; MCNEILL, A.; GIBBS, D.J. (Ed.). **Stable isotope techniques in the study of biological process and functioning of ecosystems.** Dordrecht: Kluwer, 2001. p.19-36. (Current Plant Science and Biotechnology in Agriculture, 40).

PEREIRA, A.L. Isótopos estáveis em estudos ecológicos: métodos, aplicações e perspectivas. **Revista Biociências**, Taubaté, v.13, n.1-2, p.16-27, 2007.

RATCLIFFE, R.G.; SHACHAR-HILL, Y. Measuring multiple fluxes through plant metabolic networks. **Plant Journal**, Oxford, n.45, p.490-511, 2006. **(1)**

RHOADES, J.D.; LOVEDAY, J. Salinity in irrigated agriculture. In: STEWART, B. A.; NIELSEN, D. R. (Eds.). Irrigation of Agricultural Crops. Agronomy Monograph n.30, SSSA, Madison, p. 1089–1142, 1990.

SMITH, B.N.; EPSTEIN, S. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. **Plant Physiology**, Rockville, v.46, p.738-742, 1970.