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HYPOXIA TOLERANCE OF RICE VARIETIES FOR UPLAND AND IRRIGATED CULTIVATION

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ABSTRACT: Hypoxia can intensify the impacts of salinity on world rice production. Thus, understanding how the varieties of this crop behave under hypoxia can help measure the impact of selection pressure on the formation of varieties with different cultivation recommendations on tolerance to abiotic stresses. In this sense, two rice varieties, one from the irrigated system and the other from the rainfed system, were subjected to hypoxia to assess tolerance and whether it is related to specific characteristics such as changes in the pattern of ionic homeostasis or even reduction of K^+ efflux, as suggested for some research. The results showed greater tolerance to hypoxia for var. BRS Esmeralda, recommended for rainfed cultivation, than for a var. São Francisco recommended for irrigated cultivation. The severe hypoxia increased the accumulation of Na^+ in the stems and roots of the var. São Francisco. Moreover, despite the K^+ content was preserved in leaves and roots under severe hypoxia in both varieties; a K^+ efflux by severe hypoxia was noticed in the São Francisco' stems.

KEYWORDS: Ion accumulation, abiotic stress, *Oryza sativa* L.

TOLERÂNCIA À HIPÓXIA DE VARIEDADES DE ARROZ PARA CULTIVO DE SEQUEIRO E IRRIGADO

RESUMO: A hipóxia pode agravar os impactos provocados pela salinidade na produção mundial de arroz. Desta forma, compreender como as variedades desta cultura se comportam sob hipóxia pode ajudar a dimensionar o impacto da pressão de seleção para a formação de variedades com distintas recomendações de cultivo sobre a tolerância aos estresses abióticos.

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Neste sentido, duas variedades de arroz, uma de sistema irrigado e outra de sequeiro, foram submetidas à hipóxia para avaliar a tolerância, e se ela está relacionada às características específicas como alteração do padrão de homeostase iônica ou mesmo redução do efluxo de K^+, como sugerido por algumas pesquisas. Os resultados apontaram maior tolerância à hipoxia para a var. BRS Esmeralda, recomendada para cultivo de sequeiro, que para a var. São Francisco, recomendada para cultivo de sequeiro. Também, a hipóxia severa aumentou o acúmulo de Na^+ nas hastes e raízes da var. São Francisco. Além disso, apesar do conteúdo de K^+ ter sido preservado nas folhas e raízes sob hipóxia severa em ambas as variedades um efluxo de K^+ pela hipóxia severa foi notado nos caules da var. São Francisco.

PALAVRAS-CHAVE: Acúmulo de íons, estresse abiótico, *Oryza sativa* L.

INTRODUCTION

The plants are subject to several conditions of biotic and abiotic stresses, being quite common the stresses caused by the salinity's soil or irrigation water and by flooding, which causes hypoxia conditions. These stresses significantly impact the productivity and availability of rice-growing areas worldwide (PANDEY et al., 2017). In Brazil, rice varieties are developed for cultivation under dry or irrigated conditions. Most of the rice produced in the country is cultivated under irrigation, subject to salinity and hypoxia. Furthermore, it is known that about 60 to 80 million hectares of land are affected, to some extent, by flooding combined with salt stress (FAO, 2011). This situation increases production risk since hypoxia severe (very close to anoxia) causes critical damage to photosynthesis and ion homeostasis disturb, increasing the susceptibility to salt even in species adapted to hypoxia as rice (LOPES et al., 2020). It is essential to highlight that cytosolic potassium homeostasis under stress has been identified as critical for hypoxia tolerance. K^+ efflux's restriction in mutant Arabidopsis plants (gork1-1) caused high tolerance to hypoxia stress (WANG et al., 2016). However, evidence suggests that stress-induced K^+ efflux is also essential in mediating plant growth and development under hostile conditions (SHABALA, 2017). Thus, given the above, it was intended to assess whether the hypoxia tolerance is related to the type of cultivation technique for which the rice varieties were selected and the K^+ efflux reduction.

MATERIAL AND METHODS

Rice seeds of varieties São Francisco (SF) and BRS Esmeralda (ES) were provided by the Brazilian Company of Agricultural Research (Embrapa). The São Francisco variety was developed for irrigated cultivation while BRS Esmeralda for rainfed or upland cultivation (CASTRO et al., 2014; EMBRAPA, 2013). Seeds were sown in germitest paper and placed in the BOD chamber under controlled conditions: 30 °C, relative humidity greater than 90%, and a 12-h photoperiod. The seedlings grew in the BOD chamber until the coleoptile reached approximately 5 cm, which took ten days. Three ten-day-old seedlings were transferred to each plastic pot (3.0 L) containing modified Clark's nutrient solution (CLARK, 1975) Fe–EDTA concentration of 0.076 mM. The nutrient solution had 5.5 ppm or mg L^{-1} of dissolved oxygen (DO). The experiments were carried out under greenhouse conditions: average air temperature of 33 °C during the day and 27 ºC at night, average air relative humidity of 75%, and a 12-h photoperiod. After growing in the greenhouse, plants with twenty-five days old of both varieties were submitted to hypoxia treatments. The experiment was performed in a complete randomized design, in a 2 × 3 factorial scheme, composed of two varieties (SF and ES) and three different hypoxia conditions [severe (DO < 3.5 ppm or 109.4 µM or 0.28 kPa), moderate (DO ≅ 5.5 ppm or 171.9 µM or 0.44 kPa) and slight (DO ≅ 7.5 ppm or 234.4 µM or 0.6 kPa)]. The hypoxia treatments were applied as Lopes et al. (2020). The data were subjected to the Shapiro-Wilk test before being subjected to variance analysis (two-way ANOVA). The mean treatment values were separated by Sidak or Tukey's test ($p < 0.05$), using the GraphPad Prism 8 program.

The samples were collected ten days after the application of hypoxia. The plant material was divided into leaves, stems, and roots. After measuring the fresh mass, the material kiln-dried in a forced-air circulation oven at 60 °C for 48 h for determining the leaves, stems, and root dry mass. The inorganic ions (Na^+, K^+, and Cl^-) were extracted from leaves, stem, and roots, according to Cataldo et al. (1975). For this, 50 mg of the dry mass was finely powdered and homogenized with 5.0 mL of deionized water for one hour at 75 ºC. The tubes containing the homogenate were vigorously shaken every 15 min and then centrifuged at 3,000 x g for 10 min. The obtained supernatant was filtered on filter paper and stored at -20 °C. It was then used for Na^+ and K^+ ions determination using flame photometry (Micronal®, model B462, Brazil), according to Malavolta et al. (1989), being carried out one read for each ion. The Cl^- content was determined according to the colorimetric method of Gaines et al. (1984) with the aid of a spectrophotometer set at 460 nm and a standard curve with NaCl.

RESULTS AND DISCUSSION

The slight hypoxia did not allow a higher accumulation of dry leaf mass in both varieties than moderate hypoxia (Fig. 1a). Severe hypoxia did not restrict dry mass accumulation in any part of the SF and ES varieties (Fig. 1a-c). It is notorious that SF invests more in the accumulation of dry mass in the leaves regardless of the level of hypoxia, while ES presents a more significant accumulation in the stems and roots. The ES does not significantly differ in the shoot length due to the alteration in the growth medium's oxygenation. Still, SF presented a reduction in the shoot length when compared to regular rice cultivation conditions, that is, moderate hypoxia (Fig. 1d). SF showed root shortening despite not reducing their roots' dry mass under severe hypoxia (Fig. 1e). Thus, the more significant oxygen restriction with severe hypoxia caused changes in the growth pattern of this variety and, on the other hand, the better oxygenation of the growth medium is not capable of bringing significant improvements for the growth of rice plants, as observed in another rice variety by Lopes et al. (2020). A higher tolerance in ES can be attributed to the non-shortening of the plant observed in SF. So despite the ES having suffered high selection pressure for cultivation in highlands, not subject to hypoxia, it preserved efficient adaptive mechanisms to deal with even severe hypoxia.

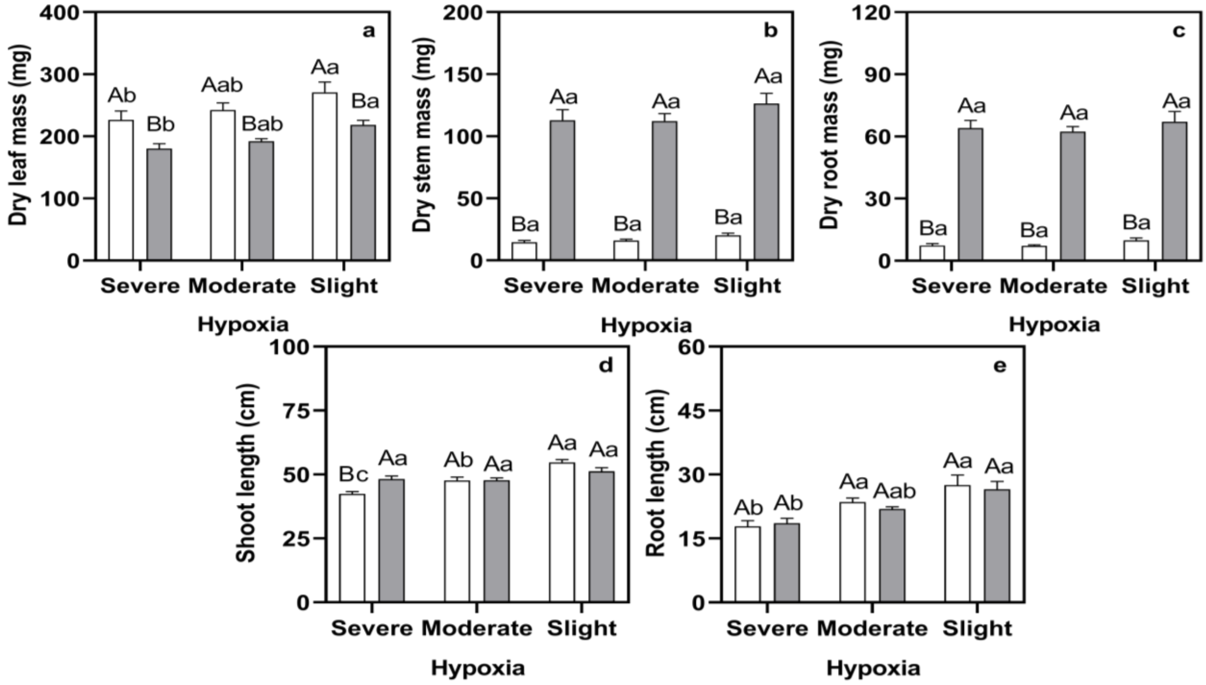

Figure 1. Growth analysis of rice plants var. São Francisco (*white bars*) or BRS Esmeralda (*grey bars*) under severe (109.4 μM O_2), moderate (171.9 μM O_2), or slight (234.4 μM O_2) hypoxia conditions for ten days. Dry mass of leaves (**a**), stems (**b**), roots (**c**), and shoot (**d**) and root (**e**) length. For each variable, the capital letters and lowercase letters compare the variety and hypoxia treatments, respectively. All subfigures exhibit a significant interaction between treatments except **a**, **b**, and **c**, according to *F*-test ($p < 0.05$). Error bars represent ± standard error and means represent n = 6.

In SF and ES, greater oxygenation (slight hypoxia) promotes less Na^+ accumulation in the leaves (Fig. 2a). Severe hypoxia increased the Na^+ accumulation in the stems and roots of the SF, while the Na^+ levels were not altered in these organs of ES plants by the levels of hypoxia (Fig. 2b, c). SF plants accumulated more Na^+ than ES in the leaves under severe and slight hypoxia and in the roots under severe hypoxia but had fewer Na^+ than ES in the stem only under moderate hypoxia (Fig 2a-c). SF accumulates more Cl^- in the leaves under slight hypoxia, and ES reduces the accumulation of Cl^- in the stem and increases in the roots under severe and slight hypoxia (Fig. 2d-f). SF plants accumulate more Cl^- than ES in the leaves under slight hypoxia and in the roots under moderate hypoxia, but have less Cl^- than ES in the stem under moderate hypoxia and the roots under severe hypoxia. Solely the slight hypoxia increases the K^+ accumulation in the SF leaves and in the ES leaves and stems (Fig. 2g, h). Severe hypoxia did not change the K^+ content in the SF and ES plants, except for reducing its content in the SF stems (Fig. 2g-i). No significant changes in the K^+ content occurred in the roots of both rice varieties by hypoxia levels. However, the K^+ content in ES was higher than in SF at any level of hypoxia. The varieties showed similar K^+ levels in the leaves, regardless of the hypoxia level. On the other hand, the more significant K^+ accumulation in SF's stems than in ES under moderate hypoxia does not remain under severe and slight hypoxia.

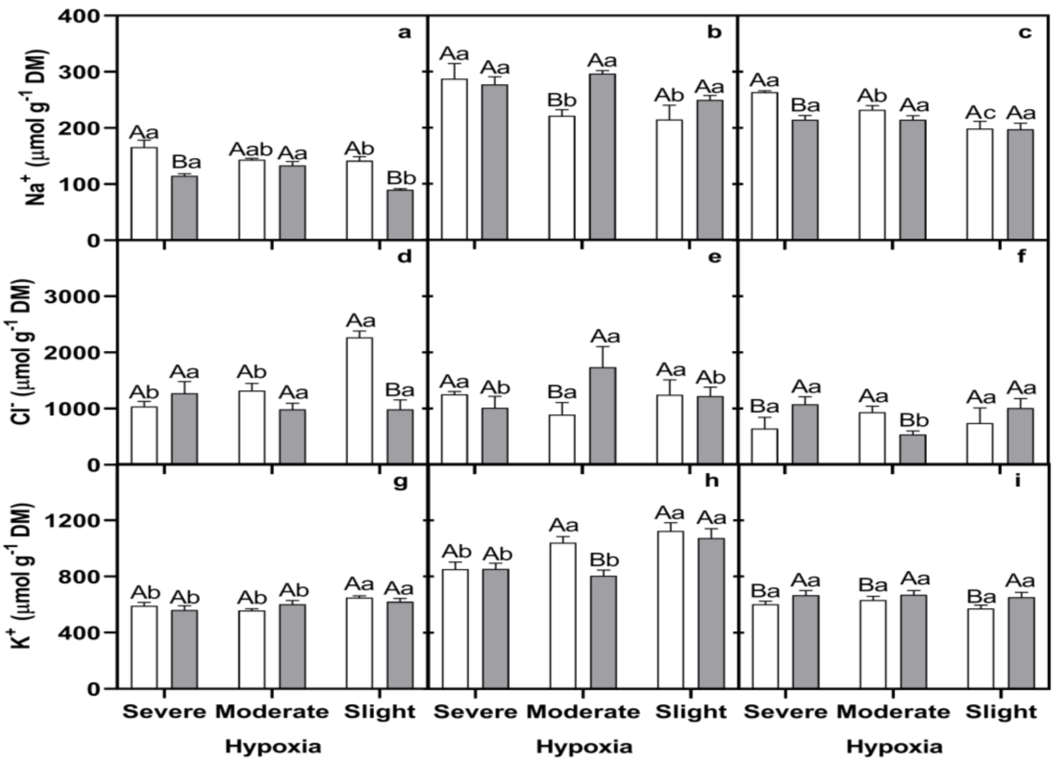

Figure 2. Inorganic ions of rice plants var. São Francisco (*white bars*) or BRS Esmeralda (*grey bars*) under severe (109.4 µM O_2), moderate (171.9 µM O_2), or slight (234.4 µM O_2) hypoxia conditions for ten days. Na^+ content in leaves (**a**), stems (**b**), and roots (**c**); Cl^- content in leaves (**d**), stems (**e**), and roots (**f**); and K^+ content in leaves (**g**), stems (**h**), and roots (**i**). All subfigures exhibit a significant interaction between treatments except **g** and **i**, according to *F*-test ($p < 0.05$). For more details, see fig. 1.

CONCLUSIONS

The varieties showed no damage in the accumulation of mass, but severe hypoxia caused the plants' shortening only of the SF variety. Thus, ES showed greater tolerance to hypoxia than SF. Severe hypoxia increased the Na^+ accumulation in the stems and roots of the SF. K^+ content was preserved in the leaves and roots under severe hypoxia in both varieties so that a K^+ efflux by severe hypoxia was noticed only in the SF's stems.

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