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PLANT ABIOTIC STRESS TOLERANCE

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Fábio Steiner
(Organizador)

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APRESENTAÇÃO

A obra "Plant Abiotic Stress Tolerance", uma publicação da Pantanal Editora, apresenta, em seus 9 capítulos, uma ampla gama de assuntos sobre os recentes avanços e conhecimentos científicos nas áreas de ecofisiologia da produção vegetal e conservação dos recursos naturais e meio ambiente. Os temas abordados mostram algumas das ferramentas atuais que permitem o incremento da produção de alimentos, a melhoria da qualidade de vida da população, e a preservação e a sustentabilidade dos recursos disponíveis no planeta. A obra, vem a materializar o anseio da Editora Pantanal na divulgação de resultados e conhecimentos, que contribuem de modo direto no desenvolvimento humano.

Nas últimas décadas, a produção de alimentos tem sido frequentemente limitada por inúmeros fatores de estresse abióticos, dentre os quais, podemos citar a baixa disponibilidade de água (deficiência hídrica), temperaturas extremas (frio, geadas, calor e fogo), salinidade, deficiência de nutrientes minerais e toxicidade. Esses fatores são responsáveis por consideráveis perdas econômicas tanto para os pequenos agricultores quanto para os produtores de commodities como a cultura da soja, entre outras. Além disso, estes danos podem ser potencialmente agravados pelos efeitos das recentes mudanças climáticas globais, sendo, portanto, a sua mitigação um grande desafio para a comunidade científica. O foco principal das pesquisas abordadas neste e-book é compreender os mecanismos de defesa/tolerância dos estresses abióticos em plantas e apresentar tecnologias e práticas de manejo que possibilitem o aumento da tolerância das plantas a esses estresses abióticos.

Temas associados à identificação de cultivares de soja tolerantes à seca e o manejo da salinidade e da restrição hídrica nas culturas de soja, amendoim e pepino são abordados. A tolerância de plantas de pinhão-manso a toxicidade do alumínio (Al3+), a tolerância de quatro espécies hortícolas ao estresse térmico causado por altas temperaturas e a tolerância de mutantes de trigo ao estresse salino também é sugerido. Na área de recursos naturais é mostrado os efeitos fitotóxicos dos metais pesados nas plantas cultivadas e o estresse ambiental causado pelo fogo na região do Cerrado. Portanto, esses conhecimentos irão agregar muito aos seus leitores que procuram promover melhorias quantitativas e qualitativas na produção de alimentos e, ou melhorar a qualidade de vida da sociedade. Sempre em busca da sustentabilidade do planeta.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas áreas de ecofisiologia da produção vegetal e conservação dos recursos naturais e meio ambiente, os agradecimentos do Organizador e da Pantanal Editora.

Por fim, esperamos que este e-book possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias. Assim, garantir uma difusão de conhecimento fácil, rápido para a sociedade.

Fábio Steiner

PRESENTATION

The eBook "Plant Abiotic Stress Tolerance", a publication by Pantanal Editora, presents in its 9 chapters a wide range of questions about recent advances and scientific knowledge in the areas of ecophysiology of plant production and conservation of natural resources and the environment. The topics presented show some of the current tools that allow the increase in food production, the improvement of quality of life in people's and the preservation and sustainability of the resources available on the planet. This eBook materializes Editora Pantanal's desire to disseminate results and knowledge, which directly contribute to the development of society.

In the last decades, food production has often been limited by numerous abiotic stress factors, among which, we can mention the low availability of water (water deficit), extreme temperatures (cold, frosts, heat and fire), salinity, mineral nutrient deficiency and toxicity. These factors are responsible for considerable economic losses, both for small farmers and for producers of commodities such as soybean, among others. In addition, these damages can potentially be aggravated by the effects of recent global climate changes, and therefore, mitigating these damages is a major challenge for the scientific community. The main objective of the research presented in this e-book is to understand the defense or tolerance mechanisms of abiotic stresses in plants and to present technologies and management practices that enable greater tolerance of plants to these abiotic stresses.

Topics associated with the identification of drought-tolerant soybean cultivars and the management of salinity and water restriction in soybean, peanut and cucumber crops are presented. The tolerance of physic nut plants to aluminum toxicity (Al^{3+}), the tolerance of four vegetable species to heat stress caused by high temperatures and the tolerance of wheat mutants to salt stress is also suggested. In the area of natural resources, the phytotoxic effects of heavy metals on plant growth and the environmental stress caused by fire in the Cerrado region are shown. Therefore, this knowledge can add much to its readers who seek to promote quantitative and qualitative improvements in food production and, or improve the quality of life in society. Always in search of the planet's sustainability.

To the authors of the chapters, for their dedication and efforts, that made this eBook possible, which exposes the recent scientific and technological advances in the areas of ecophysiology of plant production and conservation of natural resources and the environment, thanks to the Organizer and Pantanal Editora.

Finally, we hope that this e-book can collaborate and instigate more students and researchers in the constant search for new technologies. Thus, ensuring an easy and quick dissemination of knowledge to society.

Fábio Steiner

PRESENTACIÓN

El trabajo "Plant Abiotic Stress Tolerance", publicación de Pantanal Editora, presenta, en sus 9 capítulos, una amplia gama de temas sobre avances recientes y conocimientos científicos en las áreas de ecofisiología de la producción vegetal y conservación de los recursos naturales y el medio ambiente. Los temas tratados muestran algunas de las herramientas actuales que permiten el aumento de la producción de alimentos, la mejora de la calidad de vida de la población y la preservación y sostenibilidad de los recursos disponibles en el planeta. El trabajo materializa el afán de Editora Pantanal por difundir resultados y conocimientos, que contribuyan directamente al desarrollo humano.

En las últimas décadas, la producción de alimentos se ha visto a menudo limitada por numerosos factores de estrés abiótico, entre los que podemos mencionar la baja disponibilidad de agua (deficiencia de agua), temperaturas extremas (frío, heladas, calor y fuego), salinidad, deficiência, nutrientes minerales y toxicidad. Estos factores son responsables de considerables pérdidas económicas tanto para los pequeños agricultores como para los productores de commodities como la soja, entre otros. Además, estos daños pueden verse potencialmente agravados por los efectos de los cambios climáticos globales recientes y, por lo tanto, mitigarlos es un desafío importante para la comunidad científica. El foco principal de las investigaciones cubiertas en este libro electrónico es comprender los mecanismos de defensa / tolerancia contra el estrés abiótico en las plantas y presentar tecnologías y prácticas de manejo que permitan aumentar la tolerancia de las plantas a estos estreses abióticos.

Se abordan temas relacionados con la identificación de cultivares de soja tolerantes a la sequía y el manejo de la salinidad y la restricción hídrica en cultivos de soja, maní y pepino. También se sugiere la tolerancia de las plantas de frutos secos a la toxicidad del aluminio (Al^{3+}), la tolerancia de cuatro especies hortícolas al estrés por calor causado por las altas temperaturas y la tolerancia de los mutantes del trigo al estrés por sal. El área de recursos naturales muestra los efectos fitotóxicos de los metales pesados en las plantas cultivadas y el estrés ambiental causado por los incendios en la región del Cerrado. Por tanto, este conocimiento aportará mucho a sus lectores que buscan promover mejoras cuantitativas y cualitativas en la producción de alimentos y, o mejorar la calidad de vida en la sociedad siempre en busca de la sostenibilidad del planeta.

A los autores de los distintos capítulos, por su dedicación y esfuerzo irrestricto, que hizo posible este trabajo, que retrata los recientes avances científicos y tecnológicos en las áreas de ecofisiología de la producción vegetal y conservación de los recursos naturales y el medio ambiente, gracias a la Organización y a Pantanal Editora.

Finalmente, esperamos que este libro electrónico pueda colaborar e instigar a más estudiantes e investigadores en la búsqueda constante de nuevas tecnologías. De esta forma, se garantiza una fácil y rápida difusión del conocimiento a la sociedad.

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Capítulo III

Co-inoculation of peanut with *Bradyrhizobium* and *Azospirillum* promotes greater tolerance to drought

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the main oil crops in the world. Brazil is the second largest producer and exporter of peanuts in Latin America with 470 thousand tons, surpassed only by Argentina, which annually produces around 1.0 million tons (Conab, 2020). Peanut cultivation has been an excellent diversification alternative for family farmers, especially due to its multiple uses and high energy and nutritional value (Nakagawa; Rosolem, 2011). However, the occurrence of water deficiency has often limited the development and productivity of culture in all producing regions of Brazil (Ferrari-Neto et al., 2012; Pereira et al., 2012; Martins, 2013). Therefore, studies that aim to evaluate management strategies that mitigate the negative effects of water restriction are important for agricultural research.

Drought results in the reduction of transpiration rate, stomatal conductance, photosynthetic rate (Vieira et al. 2017), and may lead to changes in the root growth rate, initiation rate of the reproductive structures and the rate of leaf initiation and expansion (Silva et al., 2019). Also, drought stress alters the activity of the nitrogen and carbon metabolism enzymes, as well as cause changes in the antioxidant levels (Mantovani et al. 2015). An early response to drought stress is a reduction in leaf area and plant growth rate, which allows plants to reduce their transpiration rate, thus increasing water use efficiency (WUE) (Xu et al. 2010). Plant response to drought stress includes morphological and biochemical changes. However, this response depends on several factors such as developmental stage, severity, and duration of stress and cultivar genetics.

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Many factors can affect plant responses to drought stress, such as genotype, stage of plant development, severity, and duration of water restriction (Patanè et al., 2013; Naghavi et al., 2013; Petrovic et al ., 2016), among other environmental factors. The peanut development stages most sensitive to water stress are the plant emergence and pod formation (fruiting) phases (Ferrari-Neto et al., 2012). The occurrence of water restriction during these two periods of development results, respectively, in the failure to establish the plant stand and in reducing the filling of grains, which compromises the yield of crop pods (Nakagawa; Rosolem, 2011).

Among the management practices which can be adopted to mitigate the deleterious effects of drought stress, the use of plant growth-promoting rhizobacteria (PGPR) along with rhizobia has been increasing in recent years (Gusain et al. 2015; Agami et al. 2016; Vurukonda et al., 2016; Silva et al., 2019; *Souza et al., 2020*). The PGPR may promote plant growth by regulating plant hormones, improve nutrition acquisition and symbiotic nitrogen fixation, siderophore production, and enhance the antioxidant system (Perrig et al., 2007; Hungria et al., 2013; Inagaki et al., 2014; Chibeba et al., 2015; Fukami et al., 2018). Also, some PGPR may also infer more specific plant growth-promoting traits, such as drought tolerance (Naveed et al. 2014; Agami et al. 2016; Silva et al., 2019).

Bacteria of the genus *Azospirillum* are, certainly, the most employed PGPR in Brazil and worldwide (Hungria e Nogueira, 2013; Agami et al. 2016; Vurukonda et al., 2016; Silva et al., 2017; Silva et al., 2019; *Souza et al., 2020*), but the beneficial effects of the association of *Azospirillum* with rhizobia, when both are applied in the leguminous plants, are still incipient and contradictory (Chibeba et al. 2015; Fipke et al. 2016; Zuffo et al. 2016; Bulegon et al. 2017). Recent research results have shown that co-inoculation of rhizobia and *Azospirillum brasilense* improves seed germination, plant growth, root branching and nodulation of soybean plants (Bulegon et al. 2017; Silva et al., 2019). Furthermore, *Azospirillum* can stimulate root hair formation and root growth, increasing the volume of soil explored by the root system, which can help plants to overcome environmental stresses (Chibeba et al. 2015; Bulegon et al. 2017; Fukami et al., 2018). However, the effects of the co-inoculation of these rhizobacteria in improving the tolerance of peanut crops to water deficiency are still incipient and inconclusive.

This study was designed to investigate the effectiveness of inoculation with *Bradyrhizobium japonicum* and *Azospirillum brasilense* either alone or in combination on the growth and tolerance of peanut plants [*Arachis hypogaea* L., cv. RUNNER IAC 886] to drought stress.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions in Cassilândia, MS, Brazil (19°05'29" S, 51°28'29" W, and altitude of 535 m), in 12-L plastic pots, where the midday photosynthetic photon flux density was 980 μmol m^{-2} s^{-1}, the mean air temperature was 26.8 ºC during the day and was 23.4 ºC during the night, and mean relative humidity was 68%.

The soil used in the experiments was a sandy Arenic Entisol collected from the plow layer in a savanna area with 130 g kg^{-1} of clay, 30 g kg^{-1} of silt, and 840 g kg^{-1} of sand. The soil had the following properties: pH in $CaCl_2$ = 4.5; P (Mehlich-1) = 4.8 mg dm^{-3}; K (Mehlich-1) = 31 mg dm^{-3}; Al^{3+}, Ca^{2+}, Mg^{2+}, H^{+}+Al^{3+} = 0.55, 1.30, 0.40, 3.70 $cmol_c$ dm^{-3}, respectively; CEC = 5.48 $cmol_c$ dm^{-3}; base saturation = 32%; and organic matter = 6.5 g dm^{-3}. The field capacity, or its equivalent for soils in pots, the "pot capacity", was measured under free draining conditions using the decrease rate of water content of 0.1 g kg^{-1} day^{-1} as previously recommended by Casaroli and Lier (2008), and the soil moisture content at pot capacity (PC) was 210 g kg^{-1}.

Lime (36% CaO, 14% MgO and ECC 85%) was incorporated into each pot at the rate of 820 mg dm^{-3} of soil to raise soil base saturation to 60% (Sousa; Lobato, 2004). The limed soil was then moistened and maintained for 40 days with water content close to pot capacity. After this period, soil was fertilized with 30 mg dm^{-3} of N (urea), 250 mg dm^{-3} of P (triple superphosphate), 100 mg dm^{-3} of K (potassium chloride), 15 mg dm^{-3} of S (gypsum), 2 mg dm^{-3} of Cu (copper sulfate), 2 mg dm^{-3} of Zn (zinc sulfate), 1 mg dm^{-3} of Mo (ammonium molybdate), and then subjected to peanut cropping.

The experiment was arranged in a randomized block design, using four inoculation treatments [control (uninoculated), inoculation with *Bradyrhizobium japonicum*, inoculation with *Azospirillum brasilense*; co-inoculation with *B. japonicum* and *A. brasilense*] and three levels of drought stress [100% of PC (well-watered control), 50% of PC (moderate stress) and 25% of PC (severe stress)], considering a factorial arrangement (4 × 3) with four replicates.

A total of 96 pots were used – 8 pots per treatment. Four replicates were used for destructive samplings, including leaf area (LA) and dry matter production of plants after 18 days of drought stress. The other four pots were used for the measurement of leaf relative water content and electrolyte leakage from cells during the 18 days of drought stress and after 3 day's recovery of plants in well-watered conditions. Each plastic pot was filled with 14 kg (or 10.5 dm^3) of air-dried soil and, sieved in a 4.0 mm mesh.

Seed inoculation with *Bradyrhizobium japonicum* was carried out with the commercial liquid inoculant Simbiose Nod Soja® (Simbiose: Biological Agrotechnology) containing the SEMIA 5079 and SEMIA 5080 strains (minimum concentration of 7.2 × 10^9 viable cells per mL), at a rate of 3 mL kg^{-1} of seed. For the inoculation with *Azospirillum brasilense*, the commercial liquid inoculant AzoTotal® (Total Biotechnology) containing the AbV_5 and AbV_6 strains (minimum concentration of 2.0 × 10^8 viable cells per mL) was used, at a rate of 4 mL kg^{-1} of seed. The co-inoculation was performed by mixing the two rhizobacteria at the same proportions used when inoculated alone, that is, 3 mL of inoculant containing *B. japonicum* + 4 mL of inoculant containing *A. brasilense* per kilogram of seed. The amount of inoculants used were added to a solution containing 2 mL kg^{-1} of additive for inoculant Protege® TS (Total Biotecnologia), and then both products (inoculant + additive) were applied to the seeds. The inoculant additive consists of active metabolites of bacteria, sugar complex and encapsulating biopolymers, and has the purpose of improving the protection and viability of the bacteria on the seeds.

Seven peanut seeds from cultivar RUNNER IAC 886 previously inoculated with the respective treatments were sown in each plastic pot, and seven days after seedling emergence, seedlings were thinned to two plants per pot. The peanut cultivar used in the experiment has a creeping growth habit, 100-grain mass of 48 to 60 g, and an average cycle ranging from 125 to 135 days. Until the beginning of peg development (R2 growth stage), the soil water content was maintained at pot capacity (210 g kg^{-1}) with daily irrigation. Posteriorly, the plants were divided into three groups of water regimes [well-watered control (100% of PC), moderate stress (50% of PC) and severe stress (25% of PC)]. The plants were kept under different water regime for 18 days and evaluated by the third day after rehydration (well-watered conditions). In a previous trial, a three-day period was sufficient for the full recovery of peanut plants subjected to severe drought stress. The soil water content was monitored daily at 9:00 h and 15:00 h using humidity sensors installed at 12 cm depth. The soil moisture content (SMC, g kg^{-1}) during the 18 days of exposure to drought stress and 3-days recovery of the peanut plants under well-watered conditions was determined by the gravimetric method as described by Embrapa (1997). The effect of water stress on the growth of peanut plants is shown in Figure 1.

The water status of the plants was determined by the leaf relative water content (RWC) at 1, 3, 6, 9, 12, 15 and 18 days after starting the drought stress and at 3 days after recovery. Twenty leaf discs of 8.5 mm diameter were collected at 5:00 h (pre-dawn) from two pot plants. RWC was calculated according to the equation: RWC (%) = [(FW – DW)/(TW – DW)] × 100, where FW is the fresh weight, DW is the dry weight, after drying in the oven at 60 °C for 48 h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 6 h at 25 °C.

Leaf membrane stability index (MSI) was assessed after 18 days of drought stress and 3-d recovery as described by Lutts et al. (1996). Twenty leaf discs of 8.5 mm diameter were thoroughly washed in distilled water, placed in closed tubes containing 30 mL of deionized water and incubated at 25 °C in a water bath for 6 h. Then the electrical conductivity of the solution was recorded by EC meter (C_1). Subsequently, the same samples were placed in a boiling water bath (100 °C) for 1 h, and their electrical conductivity (C_2) was also recorded after equilibration at 25 °C. The membrane stability index was calculated according to the following formula: MSI (%) = $[1 - (C_1/C_2)] \times 100$.

Figure 1. Detail of the growth of peanut plants cv. RUNNER IAC 886 coinoculated with *Bradyrhizobium japonicum* and *Azospirillum brasilense* and grown with 100% (control), 50% (moderate stress) and 25% (severe stress) of the maximum water retention capacity of the soil for 18 days. UEMS, Cassilândia, MS, Brazil, 2018. Source: The authors.

After the 18th day of exposure to drought stress, the plants were harvested and then the plant height, leaf area, root volume, and dry matter of the plant parts were measured. The plants were separated into leaves, stem, and roots, oven-dried at 65 °C for three days and then weighed. The shoot dry matter was obtained from the sum of the dry matter of the stem and leaves. The total dry matter was obtained from the sum of all plant parts (stem, leaves, and roots).

Leaf area (LA) was determined following the methodology described by Benincasa (2003). Fifteen discs were detached from basal, median, and apical leaves. Total LA was estimated using the following equation: $LA = [(A_D \times TDM_L)/DM_D]$, where A_D is the known area of the detached leaf discs, TDM_L is the total dry matter of the leaves, and DM_D is the dry matter of the detached leaf discs. Root volume (RV, cm^3 $plant^{-1}$) was determined by water displacement using a calibrated cylinder of 250 mL.

The data normality was previously tested by the Kolmogorov-Smirnov test ($p < 0.05$) and then data were submitted to analysis of variance (ANOVA), and means were compared Tukey test at the 0.05 level of confidence. All analyses were performed using Sisvar® version 5.6 software for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

RESULTS AND DISCUSSION

The inoculation with *Bradyrhizobium japonicum* and *Azospirillum brasilense* either alone or in combination did not significantly affect ($p > 0.05$) the leaf relative water content (RWC) until the 9th day of exposure of plants to water restriction (Table 1). At 12, 15 and 18 days of water restriction, plants co-inoculated with B. japonicum and A. brasilense showed higher CRA when compared to non-inoculated plants (Table 1). These results indicate that the co-inoculation with rhizobia and azospirilla mitigated the water loss of the peanut leaves during the period of exposure of the plants to drought stress. This increase in RWC may be due to the beneficial effect of plant growth-promoting rhizobacteria (PGPR) on improving the plant water status under drought conditions as reported in other crops. Inoculation of *A. brasilense* in wheat plants (*Triticum aestivum*) under drought stress-induced an increase in leaf water content, which was attributed to the production of plant hormones such as auxin by the PGPR that enhanced root growth and formation of lateral roots thereby increasing uptake of water and nutrients under water restriction (Arzanesh et al. 2011). Similarly, *maize* plants (*Zea mays*) inoculated with *A. brasilense* and *Herbaspirillum seropedicae* exhibited higher RWC in the leaf tissue and better osmoregulation in drought conditions (*Curá et al. 2017).*

Table 1. Effects of (co)inoculation with *Bradyrhizobium japonicum* and/or *Azospirillum brasilense* and drought stress levels on the leaf relative water content (RWC) of peanut (*Arachis hypogaea* L., cv. RUNNER IAC 886) at pre-dawn during 18 days of exposure to drought stress and after 3 days of recovery of the plants under non-stress conditions. UEMS, Cassilândia, MS, Brazil, 2018

Causes of variation	**Days after the imposition of drought stress**							
	1st	**3rd**	**6th**	**9th**	**12th**	**15th**	**18th**	**21st (†)**
Inoculation treatment								
Non-inoculated plants	88 a	86 a	77 a	74 a	76 b	72 b	72 b	90 a
Bradyrhizobium japonicum	88 a	85 a	80 a	76 a	80 ab	73 b	75 ab	91 a
Azospirillum brasilense	89 a	86 a	80 a	78 a	81 ab	76 a	77 a	90 a
B. japonicum + *A. brasilense*	90 a	85 a	82 a	80 a	83 a	78 a	79 a	90 a
Drought stress level								
Control (100% PC)	91 a	92 a	91 a	92 a	91 a	90 a	92 a	92 a
Moderate stress (50% PC)	88 a	85 b	79 b	80 b	78 b	79 b	76 b	89 a
Severe stress (25% PC)	87 a	78 c	70 c	62 c	57 c	53 c	50 c	90 a
CV (%)	5.31	6.28	4.87	6.34	5.86	6.12	7.83	5.61

Mean followed by distinct letters for the factors inoculation and drought stress show significant differences (Tukey test, $P \leq 0.05$). PC: pot capacity. CV: coefficient of variation. (†) Relative water content (RWC) measured after three days of recovery of the plants under non-stress conditions. Source: The authors.

The RWC of the control plants under well-watered conditions remained constant with values reaching from 90 to 92% (Table 1). Drought stress caused a decrease in the RWC of peanut plants with values reaching 85% and 76% (moderate stress) and 78% and 50% (severe stress) at 3 and 18 days, respectively (Table 1). The decrease of 42% (severe stress) in the leaf RWC at 18 days resulted in decreased turgescence, chlorosis mainly in the old leaves, and high leaf abscission rate. After rehydration, RWC reached values similar to the control plants, restoring the leaf turgescence (Table 1). Water stress adversely affects many physiological processes in plants, and the decrease in leaf relative water content is one of the first adverse effects of drought (Taiz et al., 2017).

The inoculation with *B. japonicum* and *A. brasilense* and water restriction levels significantly affects ($p < 0.05$) the leaf membrane stability index (MSI) of peanut plants (Table 2). Plants inoculated with *B. japonicum* and *A. brasilense* either *alone* or in *combination resulted in* higher MSI when compared to non-inoculated plants (Table 2). These results indicate that damage to cell membranes caused by drought was reduced by inoculation of *A. brasilense* alone or in association with rhizobia. A similar result was reported by Abbasi et al. (2013) who showed that soybean co-inoculation with *B. japonicum*, *Azotobacter chroococcum* and *A. brasilense* in drought stress conditions improved membrane integrity when compared to non-inoculated plants. This improvement in the stability of plant cell membranes with the inoculation of *A. brasilense* and rhizobia may be due to activation of the antioxidant defense system by PGPR, enhancing drought tolerance in plants (Gusain et al. 2015). According to Vurukonda et al. (2016), PGPR-mediated changes in the integrity of the cell membranes is one of the first steps towards enhanced tolerance to water deficiency.

Drought stress led to a significant decrease in membrane stability index (Table 2). The lower stability of leaf membranes observed in peanut plants under water restriction conditions indicates that cell membrane injury was increased. Under drought stress conditions, plants produce reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radicals (O_2^-), and hydroxyl radicals (OH). These ROS react with proteins, lipids and deoxyribonucleic acid causing oxidative damage and impairing the cell membrane permeability (Vurukonda et al. 2016). The damage caused by ROS on cell integrity is due to the lipid peroxidation of cellular membranes (Taiz et al., 2017).

Table 2. Effects of (co)inoculation with *Bradyrhizobium japonicum* and/or *Azospirillum brasilense* on the leaf membrane stability index (MSI) of peanut plants [*Arachis hypogaea* L., cv. RUNNER IAC 886] under non-stress conditions (control) or plants exposed to 50% pot capacity (moderate drought stress) and 25% pot capacity (severe drought stress) for 18-days and after 3-days of plant recovery under non-stress conditions. UEMS, Cassilândia-MS, 2018

Inoculation	Water restriction level			Mean
	Control (100% PC)	Moderate stress (50% PC)	Severe stress (25% PC)	
	After 18 days of water stress			
Uninoculated plants	89.20	79.18	71.12	79.83 b
Bradyrhizobium japonicum	91.32	83.51	77.58	84.10 a
Azospirillum brasilense	93.10	84.62	79.61	85.78 a
B. japonicum + *A. brasilense*	93.25	85.48	83.42	87.38 a
Mean	91.72 A	83.20 B	77.93 C	
CV (%)	9.81			
	After 3 days of plant recovery			
Uninoculated plants	89.08	81.78	78.78	83.21 b
Bradyrhizobium japonicum	89.49	88.10	86.38	88.00 a
Azospirillum brasilense	90.72	89.36	89.48	89.85 a
B. japonicum + *A. brasilense*	90.10	90.70	91.32	90.71 a
Mean	89.85 A	87.48 B	86.49 B	
CV (%)	8.34			

Mean followed by distinct lowercase letters, for inoculation treatment (in the column) or distinct uppercase letters, for the drought levels (in the line) show significant differences (Tukey test, $p \leq 0.05$). CV: coefficient of variation. Source: The authors.

The inoculation with *A. brasilense* alone or combined with *B. japonicum* resulted in greater plant height under severe stress conditions (Figure 2A). Bulegon et al. (2016) also showed that inoculation with *A. brasilense* and *B. japonicum* resulted in higher height of soybean plants. According to Fukami et al. (2018), *A. brasilense* has the ability to produce plant hormones, such as indolacetic acid (AIA) and gibberellic acid, which improve the growth rate of plants. However, these effects were not observed under conditions of adequate water availability (control) and under moderate water restriction (Figure 2A).

In control conditions, the leaf area was significantly larger in plants inoculated with *A. brasilense*, whereas under moderate and severe drought stress the inoculation of *B. japonicum* alone or in combination with *A. brasilense* did not significantly affect ($p > 0, 05$) the leaf area of peanut plants (Figure 2B). The root volume of plants under control conditions was significantly higher with the co-inoculation of *B. japonicum* and *A. brasilense*, whereas under conditions of severe drought stress the largest root volume was obtained in plants inoculated with B. japonicum alone (Figure 1C). The *A. brasilense* can promote plant growth through the production of plant hormones, mainly indole-3-acetic acid (IAA), which can help plants overcome environmental stresses, inducing the formation of lateral roots and increased growth roots (Chibeba et al., 2015; Vurukonda et al., 2016).

The inoculation with *A. brasilense* alone and combined with *B. japonicum* resulted in an increase of 21% in the shoot dry matter production of the plants under control conditions and under severe drought stress (Figure 2D). The inoculation of *A. brasilense* alone or in combination with *B. japonicum* resulted in greater production of root dry matter under conditions of severe drought stress (Figure 2E). Plants inoculated with *B. japonicum* and *A. brasilense* alone or in combination had higher total dry matter production when compared to non-inoculated plants and exposed to severe drought stress (Figure 2F).

In general, these results suggest that the inoculation of *B. japonicum* and *A. brasilense* either *alone* or in *combination* led to an increase in the growth of peanut plants grown under water restriction conditions, resulting in higher dry matter production (Figure 2). Specifically, plants co-inoculated with both bacteria produced 19% more shoot and root dry matter than non-inoculated plants (Figure 2D and 2D). These results show that the co-inoculation of *PGPR* and rhizobia has a synergistic effect on the growth of peanut plants, as reported in other crops, such as soybeans (Chibeba et al., 2015; Silva et al., 2019) and common beans (Hungary et al., 2013). Bai et al. (2003) showed that co-inoculation of soybean plants with *B. japonicum* and *Bacillus* strains provided the largest increases in shoot dry matter, root dry matter, and total biomass. Use of *Azospirillum* may *improve* effectiveness of *Bradyrhizobium* by improving the availability of N, due to symbiotic nitrogen fixation (Chibeba et al. 2015; Fipke et al. 2016) and by producing plant hormones that stimulate plant growth (Cassán et al. 2009; Curá et al. 2017; Bulegon et al. 2017) among other factors.

The plant height, leaf area, root volume and dry matter production were drastically reduced with drought stress (Figure 2). Plants exposed to severe drought stress had on average a reduction of plant height, leaf area, root volume and shoot and root dry matter of 32%, 44%,47%, 35 and 38%, respectively, when compared to plants under well-watered conditions (Figure 2). These results report the typical response of plants to drought stress usually reported in the literature (Zoz et al., 2013; Naveed et al., 2014; Curá et al., 2017; Silva et al., 2019). One of the first processes affected in response to decreased soil water availability is cell expansion, highly dependent process turgidity of the plants (Taiz et al., 2017). However, with the advancement of drought, other physiological processes are affected, with direct effects on the photoassimilates accumulated by the plant, reduction in the carbon assimilation rate and relative growth rate (Pinheiro; Chaves, 2011). As a result of these effects, there is a reduction in leaf area and dry matter production. The reduction of leaf area and leaf production occurs as a defense reaction of plants to drought, reducing transpiration rate and therefore, the water loss to the atmosphere (Taiz et al., 2017).

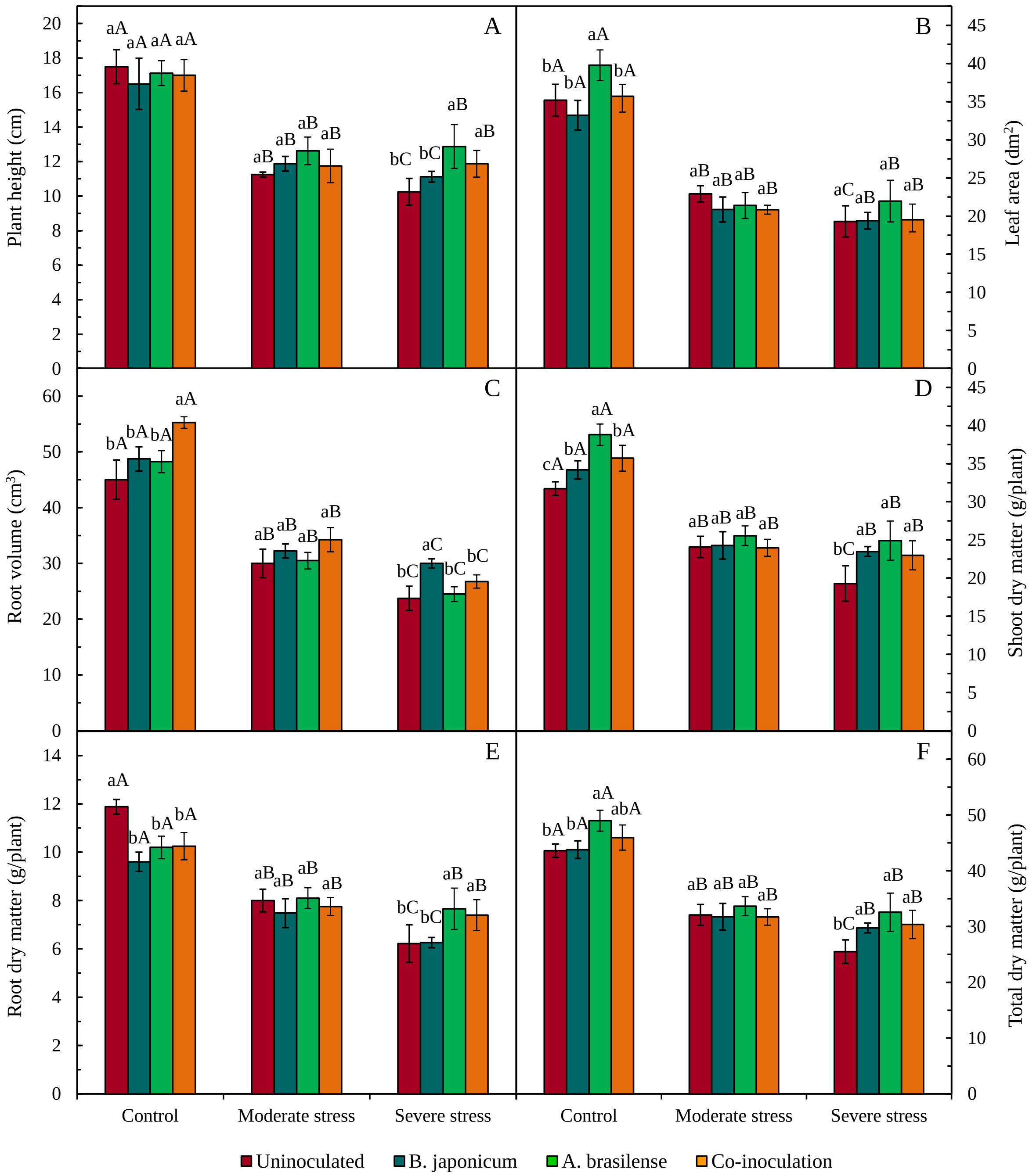

Figure 2. Effects of (co)inoculation with *Bradyrhizobium japonicum* and/or *Azospirillum brasilense* on the plant height (A), leaf area (B), root volume (C), shoot dry matter (D), root dry matter (E), and total dry matter (F) of peanut plants [*Arachis hypogaea* L., cv. RUNNER IAC] under non-stress conditions (control) or plants exposed to 50% pot capacity (moderate drought stress) and 25% pot capacity (severe drought stress) for 18 days. Bars followed by distinct lowercase letters, for inoculation treatment or distinct uppercase letters, for the drought levels show significant differences (LSD test, $p \leq 0.05$). Data refer to mean values (n = 4) ± standard error. UEMS, Cassilândia-MS, 2018. Source: The authors.

FINAL CONSIDERATIONS

The inoculation of peanut plants with *B. japonicum* and *A. brasilense* either *alone* or in *combination* improved leaf membrane stability and minimized water loss from peanut leaves when exposed to drought stress. In addition, inoculation with *A. brasilense* alone or in combination with B. japonicum resulted in higher plant height and greater root dry matter under conditions of severe drought stress. Therefore, our results suggest that inoculation with *B. japonicum* and *A. brasilense* either *alone* or in *combination* can mitigate the adverse effects of drought stress, maintaining the growth and dry matter accumulation of plants when exposed to water restriction un greenhouse conditions. Thus, important contributions from the use of biological and low-cost technologies are being made available to peanut farmers in the Cerrado region. However, the beneficial effects of the use of co-inoculation of bacteria of the genus *Bradyrhizobium* and *Azospirillum* in the cropping of peanut crop under Cerrado conditions must be confirmed with further studies under field conditions.

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