Exogenous pyruvate mitigates the detrimental effects of water stress in contrasting peanut genotypes

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ABSTRACT. Water stress triggers various cellular responses in plants, altering normal metabolic flow. Pyruvate, an important component of the glycolysis pathway, is directly involved in cell processes, triggering genes that influence drought tolerance. Research with *Arabidopsis* has shown that synthetic pyruvate relieves drought damage. We evaluated the effects of exogenous pyruvate at 100 and 50,000 µM on mitigation of drought stress in two peanut cultivars submitted to water restriction. The evaluations were based on growth, gas exchange, and photosynthesis rate analyses. In addition, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) enzymes, and free proline were also assayed. In general, exogenous application of pyruvate contributed to mitigate the effects of water stress in the cultivar IAC Caiapó (sensitive to drought), based on gas exchange and instantaneous efficiency of water use. Exogenous pyruvate contributed to restore the action of antioxidative enzymes in BR 1 (tolerant to drought), based on measures of SOD (45%), CAT (129%) and APX (60%) in stressed plants, and full recovery at 50,000 µM. When treated with proline, stress attenuation was found only at 100 µM in both cultivars; the excess seemed to have a negative effect on stressed plants, probably affecting the cell environment.

Key words: *Arachis hypogaea*; Drought tolerance; Gas exchange; Proline; Antioxidative enzymes

INTRODUCTION

Water scarcity is a climatic factor that limits crop production, causing various types of physiological and biochemical disturbances in plants. When the cell detects a water stress signal, a strategic adjustment in the defense mechanisms usually begins; a rapid physiological response differentiates tolerant from sensitive genotypes (Osakabe et al., 2014; Dutra et al., 2018).

The complex signaling pathways in response to environmental stress leads to various cellular changes, resulting in a decrease of turgor pressure in guard cells and stomatal closure. In response to drought, plants synthesize abscisic acid (ABA) that induces stomatal closure, in order to reduce transpirational water loss. This strong increase of plant ABA levels is accompanied by a major change in gene expression and in adaptive responses arising from many novel functional genes and transcription factors acting in molecular mechanisms of ABA signaling and stomatal movement (Munemasa et al., 2015). These elements activate the ion channels of the guard cells and external potassium channels in order to act on the efflux of solutes and promote stomatal closure (Osakabe et al., 2014).

All signaling, adjustment and adaptation processes demand energy for synthesis of phytohormones and others cell metabolites. Glycolysis is inserted in this context, through a sequence of reactions mediated by different enzymes in the respiratory flow of plants (Plaxton and Podestá, 2006; Rich and Maréchal, 2010). Pyruvate, a final product of glycolysis in the cytosol, is an important component of cellular metabolism, supplier of ATP and NADH in aerobic conditions. NADP-malic enzyme and pyruvate phosphate dikinase are present in C_3 and C_4 plants and are important under stress conditions. NADP-malic enzyme catalyzes the oxidative decarboxylation of L-malate to produce pyruvate, NADPH and CO_2 (Drincovich et al., 2001; Hýsková et al., 2014). In the mitochondria, each pyruvate molecule loses one carbon atom and becomes an acetyl molecule to enter the tricarboxylic acid cycle (TCA Cycle - Krebs cycle) (Plaxton and Podestá, 2006). It is a branching point for the syntheses of glucose, lactate, fatty acids, and amino acids (Rich and Maréchal, 2010; Li et al., 2014).

The import of pyruvate into mitochondria is a crucial step for both biosynthesis of organic compounds and for oxidative energy metabolism and MPC, a protein complex that negatively regulates ABA-induced guard cell signaling, are necessary for the uptake of pyruvate in the inner mitochondrial membrane (Timon-Gomez et al., 2013; Li et al., 2014; Wang et al., 2014).

Stomatal closure is mediated by MPCs, which favor plant survival, avoiding the loss of water by transpiration (Wang et al., 2014; Shen et al., 2017). In leaves, roots and flowers of *Arabidopsis*, Li et al. (2014) found robust expression of a transcript in guard cells, named *NRGA1*, that is involved in maintenance of stomatal aperture during drought stress, avoiding discontinuity of CO_2 during photosynthesis process. NRGA1/MPC is a negatively regulated protein, which contributes to alleviating the ABA effect in inhibition of inward K^+ channels during stomatal opening, favoring the pathway of anionic channels during stomatal closing. Further genetics and cellular analysis, using mutant and *Arabidopsis* transgenic plants, suggested a likely function for the MPC protein NRGA1 in plant stomatal movements and drought stress response.

Stomatal closure is one of the important responses in drought stress tolerance to reduce water loss. ABA and ROS are essential signals for these stomatal movements.

Although overproduction of ROS is related to oxidative damage, these species also act benefiting the cell system providing protection against environmental stresses (Karuppanapandian et al., 2011). NADH is required for the synthesis of ROS and consequent guard cell Ca^{2+} channel activation and stomatal closure (Timon-Gomez et al., 2013; Wang et al., 2014).

ROS production is controlled by various enzymatic and nonenzymatic antioxidative systems. Enzymatic antioxidants include CAT, POX, SOD, among others, whose compounds react with free radicals and neutralize them, overcoming the damage caused by stress (Karuppanapandian et al., 2011; Timon-Gomez et al., 2013). The accumulating of osmolytes as an adjuvant in the cellular defense processes is also widely reported. Proline is one of the most common compatible osmolytes in drought stressed plants and the accumulation in plant tissues is reliable marker for environmental stress, particularly in plants under drought stress (Hare et al., 1997; Hayat et al., 2012). However, as all defense process requires the involvement of mitochondria, it is believed that high production of endogenous pyruvate is necessary in order to minimize cell damages, which will imply in increased unfolding glucose molecules (glycolysis). Based on this hypothesis, it is possible that supplementation of exogenous pyruvate may be a substrate to supply energy in stressed plants.

Although these studies are limited, results found in literature have demonstrated pyruvate functions as a negative regulator of guard cell ABA signaling, and involvement in regulation of stomatal closure in *Commelina benghalensis and Arabidopsis* (Raghavendra et al., 1976; Li et al., 2014; Shen et al., 2017). In stressed detached leaves of *Arabidopsis*, submitted to pyruvate at 10 to 1000 μM, Shen et al., (2017) reported that the induction of stomatal closure, favored by increased pyruvate content, was dependent on the function of NADPH oxidases and ROS concentrations in guard cells. NADPH oxidases are membrane proteins that may produce ROS in plasma membrane ion channels (Keller et al., 1998). In a study carried out by Kwak et al. (2003) with *Arabidopsis*, involving functions of NADPH oxidase in ROS-dependent ABA signaling, authors provided molecular and biological evidences that ROS are rate-limiting second messengers in ABA signaling, and that NADPH oxidases function in guard cell ABA signal transduction.The peanut (*Arachis hypogaea*) is a highly appreciated oilseed and has been widely grown in both tropical and semiarid climates, to attend grain and oil markets. In dry environments, plants are constantly exposed to water stress during the cycle, affecting the yield and quality of pods, due to changes in the content of phenolic compounds and other bioactive constituents of the grains (Juliano et al., 2021). Several upright cultivars (*A. hypogaea* subsp. *fastigiata*) are short cycle and suitable for rainfed management because have tolerate to drought environments (Pereira et al., 2016; Dutra et al., 2018); the runner types (*A. hypogaea* subsp. *hypogaea*), although they are widely grown worldwide because they produce considerableoil, they are very vulnerable to water-restricted environments (Santos et al., 2012; Pereira et al., 2015). This limits the adoption these cultivars in semiarid climates.

There is evidence that exogenous application of organic compounds in plants submitted to water stress acan mitigate of the deleterious effects of drought (Karimian et al., 2015; Sadiq et al., 2017; Aziz et al., 2018). Here, we focused our research on evaluating the effects of pyruvate in peanut genotypes, hypothesizing that exogenous application could mitigate the biological damages in drought sensitive plants. In order to test this hypothesis, we chose a reference concentration, 100 uM, based on the findings of Shen et al., (2017) in

Arabidopsis, and a higher concentration (50,000 uM), to ensure increased absorption of pyruvate by the cell.

MATERIAL AND METHODS

Germplasm and growth analysis

The assay was carried out in a greenhouse (Campina Grande, PB, Brazil 07°13' S; 53°3' W), during 20 days, in the dry season (December, 2019). Ten seeds of each BR 1 (subsp. *fastigiata*, upright and tolerant to drought) and IAC Caiapó (subsp. *hypogaea*, runner and sensitive to drought), were sown in pots (750 mL, height: 13 cm, diameter: 10 cm) containing organic substrate (340 g, Basaplant Base Agro, Brazil). Each pot contained two plants. Seedlings were daily watered, keeping the substrate humidity at the level corresponding to the field capacity (Whitehead, 1966; Dane and Topp, 2002). To monitor the humidity level, we added five additional pots (750 mL, 340 g) to the experiment, with mean retention of 240 mL of water, corresponding to the level of field capacity. The substrate was moistened before twilight, with enough water to drain the excess overnight. In next morning, the amount of water retained was estimated, based on the difference between the weight of the dry and wet substrate contained in the pots. On the third day of stress, and the first day of pyruvate application, we estimated the volume of water retained in the substrate that was 35% representing 65% of deficit to reach field capacity.

After 11 d of emergence (phase V1), treatments were then differentiated: Control (C- normal watering), Stress (S- total suppression of watering), P1 and P2 (application of synthetic pyruvate (Pyruvate acid, Merck, 8.20170.0500) at 100 µM and 50,000 µM, respectively, in leaves of stressed plants. The completely randomized experimental design was adopted, with five replications. The water suppression lasted nine days, and pyruvate applications started at three days after the beginning of water stress. In this occasion, stomatal closure in stressed plants was estimated in 19.05% (*gs*: 0.42 mol H_2O m^2 s^{-1} in control, and 0.34 mol H_2O m^2s^1 in stressed plants), obtained through Infrared gas analyzer (IRGA, LCPro SD, ADC Bioscientific, UK) equipment.

The water content in substrate was estimated in 86.4% and 28.8% for control and stress treatments, respectively, based on methodology described in Uhland et al., (1951). The control of soil moisture was maintained through a daily application of 20 mL of water. This value was based on the differences between the pot weight at field capacity and after water loss during the day. The stressed treatment was achieved based on the differences between the weight of control (583 g) and stressed substrates (378 g), after 11 d of water suspension. In this occasion, the water deficit corresponded to a reduction of 65% in soil moisture, in relation to the field capacity estimated at the beginning of assay.

Pyruvate applications were performed using a cotton swab dipped in solutions (pyruvate and distilled water, v/v), sliding on a set of tetrafoliolated during 6 d, on the same leaves. During the assay, the means of temperature and relative humidity of the air (minimum and maximum) were 21.5 °C to 43 °C, and 40% to 86%.

Total number of leaves, height of main stem, dry mass of canopy and dry mass of roots were recorded for growth analysis. The tissues were dried in oven with forced air circulation, at 65 °C during 72 h.

Gas exchange and relative water content

The physiological data were collected at final of water stress, during 9:00 h to 11:00 h, using IRGA, without artificial Carbon source and with artificial light (1,200 μmol m^{-2} m^{-1}). The soil moisture recorded in control and stress treatments were 86% and 28%, respectively. The following traits were registered: net photosynthesis rate (A), intercellular CO_2 concentration (Ci), stomatal conductance (g_s) and transpiration rate (E). From these data, we estimated the instantaneous efficiency of carboxylation (IEC), based on *A/Ci* ratio, and the instantaneous efficiency of water use ($IEWU$), based on *A/E* ratio.

The relative water content (RWC) was estimated from six leaf discs collected from mature leaves located at canopy top: RWC= [(FM - DM)/(TM- DM)]x100, where FM- fresh matter, TM- turgid matter, DM- dry matter.

Antioxidant activity and free proline

A crude extract (25%) of total protein from leaves was prepared in phosphate monobasic buffer (100 mM) and EDTA (0.1 mM), pH 7.0 (Pereira et al., 2015), and quantified through Bradford (1976) method, at 595 nm. The activity of antioxidative enzymes was estimated according methodologies described in Bulbovas et al. (2005) for SOD, Azevedo et al. (1998) for CAT, and Nakano and Asada, (1981) for APX, at 560 nm, 240 nm and 290 nm, respectively. Free proline was estimated according to methodology described in Bates et al. (1973), at 520 nm.

Statistical analysis

Data were submitted to the Shapiro-Wilk normality test (Shapiro and Wilk, 1965) and to analysis of variance (ANOVA, F test) and mean classifications (Scott and Knott, 1974). The SISVAR software, version 5.6 (Ferreira, 2010) was adopted for analyses.

RESULTS AND DISCUSSION

Growth and gas exchange of peanut plants submitted to water stress with application of exogenous pyruvate

Based on the phenotypical aspect, peanut cultivars were affected differently by water stress, with visible reduction in canopy and plant height, especially in BR 1 that is an upright material and the difference becomes more visible (Figure 1). However, based on the means of growth traits, no expressive contribution was found by exogenous application of pyruvate in both cultivars. The number of leaves (Figure 2A), dry mass of canopy (Figure 2C), and dry mass of roots (Figure 2D) decreased only due to the water stress effect. The exception was found to main stem height of IAC Caiapó, that grew up approximately 37% above the mean of stressed treatment (S) (Figure 2B), whose plants was reduced by 44%, compared to height of control plants.

Figure 1. Aspect of peanut plants submitted to nine days of water stress and treated with exogenous pyruvate. A- BR 1, B- IAC Caiapó. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Pyruvate was applied to leaves of stressed plants during six days.
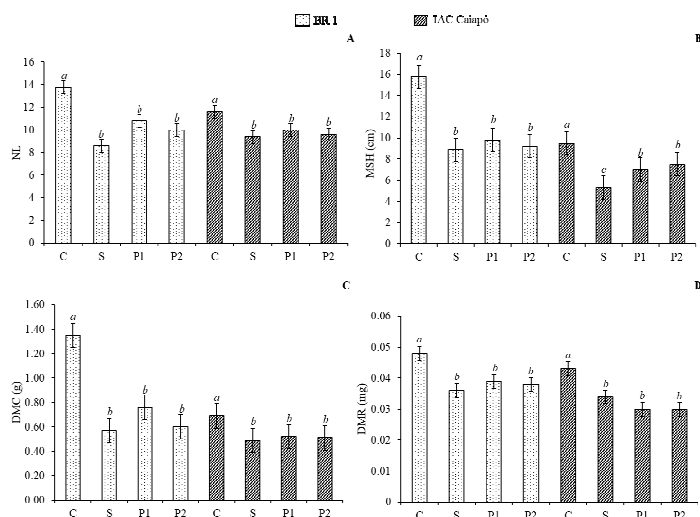

Figure 2. Growth traits of two peanut cultivars submitted to water stress and treated with exogenous pyruvate. NL- number of leaves (A), MSH- Main stem height (B), DMC- Dry mass of canopy (C), DMR- dry mass of roots (D). C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with the same letters do not differ significantly (Scott Knott test, $p \leq 0.05$).

Physiologically, the application of exogenous pyruvate was genotype-dependent, so that in BR 1, no relief of water stress was found in plants (Figure 3). Instead, in an overview, the application of exogenous pyruvate appeared to be harmful to stressed plants. In IAC Caiapó, however, exogenous pyruvate mitigated the physiological disorders, especially at highest concentration (P2, 50,000 µM). In these plants, we found an increase of 56% in *gs* (Figure 3A), and 46% in *E* (Figure 3B), compared to mean of stressed treatment, indicating attenuating effect of pyruvate in stressed plants. Compared to control (C), *gs* and *E* were reduced to 59% and 44%, respectively, in plants 9 d- water stressed, confirming the sensibility of IAC Caiapó to dry environment (Santos et al., 2012).
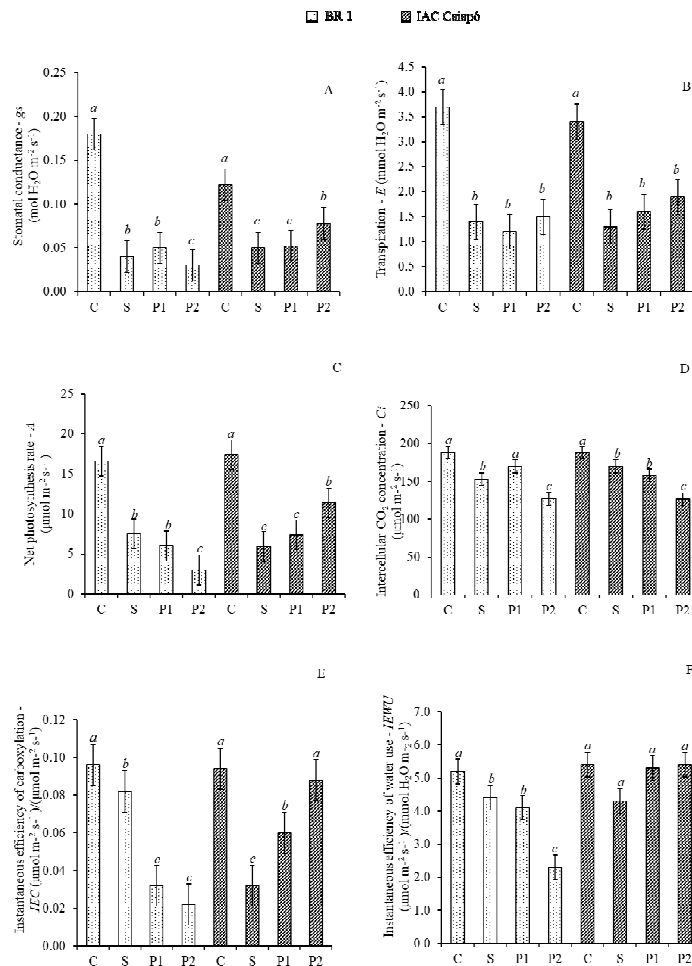

Figure 3. Gas exchanges in peanut cultivars submitted to drought and treated with exogenous pyruvate. A- Stomatal conductance (*gs*), B- Transpiration (*E*), C- Net photosynthesis rate (*A*), D- intercellular CO_2 concentration (*Ci*), E- instantaneous efficiency of carboxylation (*IEC*), F- instantaneous efficiency of water use (*IEWU*). C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

Because of attenuation of water stress on *gs* and *E* in stressed IAC Caiapó plants, the liquid photosynthesis *A* (Figure 3C) was also benefited, with an increase of 93%, in relation to stressed treatment. Based on means of control plants, we found large reductions (66%) in stressed plants, and slowed in P2 (44%).

The intercellular CO_2 concentration (*Ci*) is another relevant physiological parameter, since it is directly related to photosynthesis. Here we find the same behavior of *Ci* in P2 treatment for both cultivars, but the physiological interpretations are different, considering the values of *gs*, *E* and *A* previously reported. In IAC Caiapó, the reduction in *Ci* is a consequence of the input on carbon flow in order to favor the photosynthetic machinery, benefited by increase of *gs* (Figure 3A) and *E* (Figure 3B). Thus, the cost of *Ci* in cells of P2 plants was spent due to elevation of *A* (Figure 3C), demonstrating efficiency for CO_2 fixation, even under conditions of water deficit. In BR 1, the reduction in *Ci* appeared to be due to an unnecessary expense of energy that plants invested when interacting with a metabolite useless for the cell defense apparatus. We found that at 100 µM of pyruvate, the values of gas exchanges did not differ from the stressed treatment, indicating absence of use of product at cell level; at the highest concentration (50,000 µM), we verify that exogenous application of pyruvate was harmful to the cells, based on reduction of gas exchange traits in stressed plants (Figure 3). Although gas exchange data using BR 1 is limited in literature, the reduction of *Ci* found here in stressed plants (22%) is in agreement with that found by Dutra et al., (2018) (28%), with the same cultivar submitted to 15 d- water stress at the beginning of the reproductive phase.

The two most expressive physiological effects resulting from exogenous application of pyruvate in IAC Caiapó plants were the full relief of plants to *IEC* and *IEWU* in P2 treatment (Figure 3E, F), whose values were similar to mean of control plants. In stressed treatment, the reductions of both traits were 66% and 20%, respectively. These results open perspectives for further adoption or improvement of IAC Caiapó to environments prone to drought, taking in account that water use efficiency is a parameter of crop quality and performance of plants under water deficit (Osakabe et al., 2014). IAC Caiapo is a commercial cultivar released by IAC for tropical environment, and a valuable germplasm for oil production, as well multiple resistance to several leaf fungi (Godoy et al., 1999; Santos et 2012).

Based on a general analysis of gas exchanges of peanut cultivars showed in Figure 3, we concluded that application of exogenous pyruvate at 50,000 µM enabled favorable results to sensible IAC Caiapó, minimizing the negative effects of water stress on physiology of the plants.

Relative water content in stressed peanuts treated with pyruvate

The RWC is the most adequate measure of plant water status, since it estimates the consequence of water deficit in cells, based on effects of leaf water potential and osmotic adjustment in stressed plants. Here, we found that the application of exogenous pyruvate did not alter the water status in stressed plants of tolerant BR 1, whose results are consistent with those found in *IEWU* (Figure 3F) and reinforces the natural autonomy of this cultivar to use its physiological resources to face situations of water stress (Santos et al., 1999; Pereira et al., 2016; Dutra et al., 2018). In cultivar IAC Caiapó, however, the cells recovered the water status with application of pyruvate in both treatments, whereas in

stressed treatment the water content increased by 26% and 76%, respectively. This result possibly indicates that application of pyruvate has resulted in saving of water in peanut leaves, in order to facilitate the flow of other physiological processes.
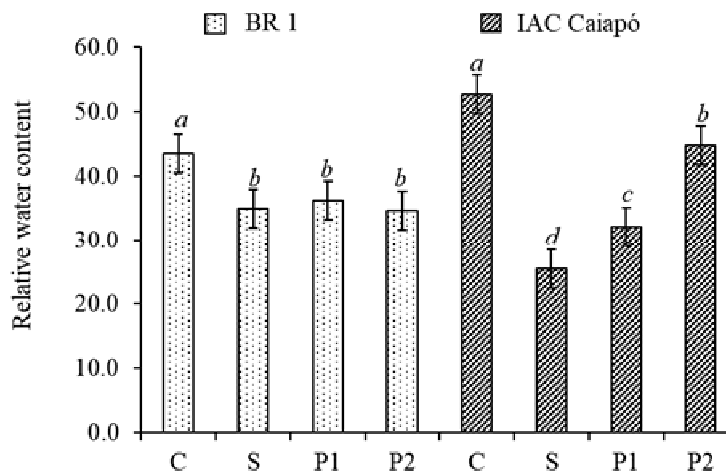

Figure 4. Relative water content in peanut cultivars submitted to water stress and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ significantly (Scott Knott test, $P \leq 0.05$).

According to Levitt (1972), plants react to drought conditions through two manners, preventing and tolerating dehydration. Plants that prevent dehydration feature functions to perform the water adjustment of cells during drought, and tolerant genotypes have tissues that support longest period of dehydration, corresponding to a low RWC. As seen in Figure 4, the reduction of RWC in stressed BR 1 was only 20%, while in IAC Caiapó was 52%, attesting the tolerance of BR 1 to the condition of water deficiency (Gomes et al., 2007; Dutra et al., 2018).

In experimental conditions adopted in this work, we found that pyruvate at 50,000 µM demonstrated action on stomatal efficiency, with reflexes on photosynthesis and water saving of IAC Caiapó. The exogenous application had a stress-relieving effect, as the stress occurred next to flowering, when plants are more vulnerable to drought (Santos et al., 2012). The brief recovery seen in *gs*, whose opening controls the balance of water loss, suggests that exogenous pyruvate contributed to better use of water available in tissues, while facing the water stress. The opposite was found in absence of pyruvate, where stressed plants were more vulnerable.

In *Arabidopsis*, Shen et al. (2017) identified novel physiological roles of pyruvate and MPC1, in the regulation of stomatal closure, in plants submitted to drought (2-3 weeks) and treated with pyruvate at 10, 100, and 1000 µM. According to authors, plants exposed to drought stress responded by elevation of ABA, which induced elevation of the cellular pyruvate content and ROS accumulation to activate slow-type anion channels, finally inducing stomatal closure. With pyruvate treatment, the stomatal aperture decreased in size under higher concentrations of pyruvate until reaching 100 µM.

It is worth pointing out here that the application of pyruvate was made only on a tetrafoliolated leaf of each plant, taking into account the direction of the translocation (source-drain) (Tanaka and Fugita, 1979). Then, the pyruvate was absorbed by the leaf cuticle and translocated to the other organs of the plant. Based on results found with IAC Caiapó, it is possible that this cultivar could benefit from the relief of cell damage caused by water stress using minor concentrations of exogenous pyruvate, although other physiological traits might not have the same tendency. This proposition could be further deepened.

Antioxidative activity and proline in plants submitted to water stress and treated with exogenous pyruvate

Mitochondria are the major intracellular source of ROS and pyruvate has a relevant role in oxidative metabolism (Li et al., 2014; Shen et al., 2017). Pyruvate regulates stomatal closure indirectly by inducing ROS production, which are cytotoxic and highly detrimental to the cellular lipids, nucleic acids and proteins (Timon-Gomez, 2013; Shen et al., 2017). Genotypes able to maintain a low steady state of ROS are better adapted to tolerate stress conditions. Plants generally scavenge and dispose of these reactive substances by the use of antioxidant defense enzymes, such as SOD, CAT, POX, APX, GR, and other associated enzymes involved in the cellular detoxification mechanism. Here, we investigate the activity of SOD, CAT and APX in BR 1 and IAC Caiapó submitted to drought and treated with pyruvate exogenous.

As seen in Figure 5A, the profile of the antioxidant enzymes in BR 1 matches fully what is expected of a drought-tolerant germplasm. An input from SOD (45%), which dismutes the superoxide radicals, was displayed right at beginning of the antioxidative process, followed by degradation of the peroxides promoted by high activity of CAT (129%) and finished by APX action (increasing of 60%) in H_2O_2 degradation. This machinery operates integrally, aiming to minimize the effect of ROS generated from water stress in cells. These inputs found in leaves of stressed plants of BR 1were reported by Pereira et al. (2012; 2015), that evaluated upright and runner genotypes submitted to drought during 7 and 10 days, respectively. The differential in this study was the mitigating action of pyruvate at 50,000 µM that totally restored the stressed plants to the normal condition, as seen in control treatment. It is possible that this action contributed to regulation of stomatal movements and reprogramming of key genes in the cell signaling network, responsible for regulating the activity of anionic channels of the guard cells.

As to IAC Caiapó (Figure 5B), the treatment of exogenous pyruvate did not contribute to mitigate the oxidative stress in plants, whose activity of the enzymes was milder, indicating slow response, often seen in sensitive genotypes (Nogueira et al., 1998).

Proline is widely cited in literature due to its influence on osmotic adjustment of plants under abiotic stress (Sivakumar et al., 2000; Alves et al., 2016). The Figure 6 displays the profile of free proline in both sensible and tolerant peanut cultivars. A

significant increase in free proline was found in stressed cultivars, on average of 317%, confirming its relevant role on regulating of the osmotic potential in cells.
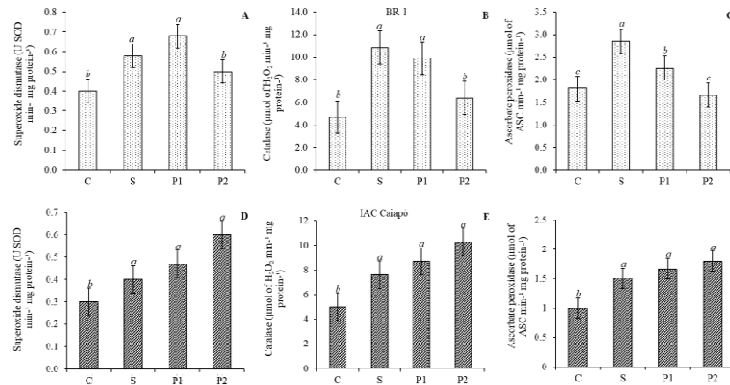

Figure 5. Antioxidative activity of SOD (A), CAT (B) and APX (C) in BR 1 and IAC Caiapó peanut cultivars submitted to water stress and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ significantly (Scott Knott test, P ≤ 0.05).
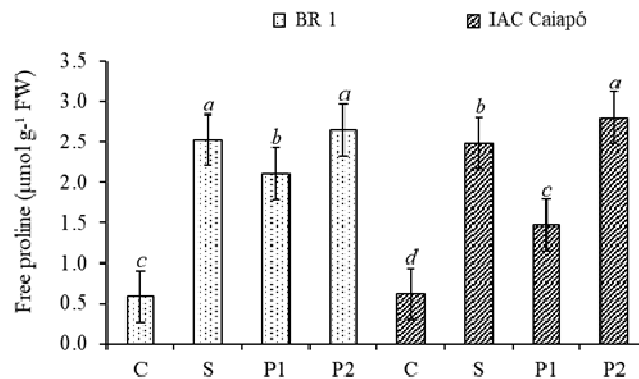

Figure 6. Free proline in peanut cultivars submitted to water deficit and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ significantly (Scott Knott test, P ≤ 0.05).

In treatments with pyruvate, however, two effects were visibly found in both cultivars: a) mild and moderate recovery of osmotic adjustment in plants at 100 µM, in the order of 16% and 40% for BR 1 and IAC Caiapó, respectively. This attenuation may be indicative of an input provided by exogenous pyruvate, generating additional energy for the Krebs Cycle machinery and electron transport chain. Additionally, the action of proline could benefits in regulation of cytosolic acidity (Sivakumar et al., 2000), and b) negative interaction between the osmolite and the endogenous pyruvate, at 50,000 µM, indicating a possible disturbance on osmotic adjustment due to excess of pyruvate in cell environment.

In drought-tolerant plants, the use of proline in cellular defense machinery is more agile, allowing a more balanced response in coping with environmental stresses (Bhatnagar-Mathur et al., 2009; Alves et al., 2016). Proline protects plants against stress by stabilizing the mitochondrial electron transport complex II and scavenging the ROS and other free radicals (Hayat et al., 2012). In the literature, several studies have demonstrated the relationship between proline and the drought tolerance response in BR 1. The proline input in leaves of plants stressed for up to 15 d ranges from 50% to 450% before blooming, indicating wide plasticity depending on the duration of stress (Pereira et al., 2015; Alves et al., 2016). Based on this behavior, breeders have considered this germplasm as a robust parent in breeding works focused on the semi-arid environment (Santos et al., 1999; Gomes et al., 2007; Dutra et al., 2018). In Brazil, a team of peanut breeders reported top lines derived from crossing between BR 1 and allotetraploids (*A. batizocoi* K9484 x *A.duranensis* SeSn 2848)$_{4x}$ showing high tolerance to drought, endorsed by the high content of proline, after submission of water stress (Dutra et al., 2018).

This work revealed information regarding relief of the deleterious effects of water stress in sensitive peanut plants , and better adjustment in the antioxidant and osmotic system of the tolerant cultivar, based on exogenous application of pyruvate. The range of concentrations adopted here were very wide because we based on the few works available in literature with exogenous pyruvate. Taking in account that a forecast of physiological and biochemical effects may occur in different species, we encourage further studies using intermediate concentrations assuming that may be possible to achieve noticeable responses in concentrations below 1000 μM.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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