MODIFICATIONS IN THE METABOLISM OF CARBOHYDRATES IN (*Coffea arabica* L. cv. SIRIEMA) SEEDLINGS UNDER DROUGHT CONDITIONS

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ABSTRACT: Understanding what mechanisms are involved in drought response in coffee plants will generate relevant information to assist breeding and/or genetic engineering to obtain new commercial varieties with drought tolerance. This research aimed at studying the effects of drought on leaf water potential, carbohydrate concentrations, and sucrose synthase and invertase activity in coffee seedlings (*Coffea arabica* Siriema), originating from a natural cross between *C. racemosa* and *C. arabica* Blue Mountain (CARVALHO et al., 2008). Seedlings were grown in a greenhouse under non-irrigated and daily irrigated conditions. Plants were evaluated every three days until they reached the permanent wilting point or, at most. 30 days after withholding water. In addition, plants under different drought conditions (as of 30 days) were irrigated and evaluated 24 and 48 hours after water application. The non-irrigated treatment prompted a significant reduction in leaf water potential, whereas re-irrigation promoted partial recovery of plant water potential. There was a significant increase in the levels of total soluble sugars and reducing sugars, both in the leaves and roots of the non-irrigated plants. The re-irrigated plants behaved like the non-irrigated plants; however, lower levels of sugars were detected in these samples. The leaves and roots of the non-irrigated plants also showed a significant reduction in starch levels. The activity of sucrose synthase and invertase, neutral and acid enzymes, increased as a result of water stress.

Index terms: *Coffea arabica*, drought, sucrose synthase, invertases, total soluble sugars.

MODIFICAÇÕES NO METABOLISMO DE CARBOIDRATOS EM MUDAS DE (*Coffea arabica* L. cv. SIRIEMA) SOB CONDIÇÕES DE DÉFICIT HÍDRICO

RESUMO: *A compreensão dos mecanismos envolvidos na resposta do cafeeiro ao déficit hídrico irá gerar informações relevantes para auxiliar no melhoramento e/ou na engenharia genética visando à obtenção de novas variedades comerciais tolerantes à seca. Objetivou-se, neste trabalho, estudar os efeitos do déficit hídrico sobre o potencial hídrico das folhas, concentrações de carboidratos e atividades da sintase da sacarose e invertase ácida em mudas de cafeeiros (Coffea arábica cv. Siriema), planta oriunda de um cruzamento natural entre C. racemosa e C. arabica cv. Blue Montain (CARVALHO et al., 2008). As plantas foram colocadas para crescimento em casa-de-vegetação, em condições não irrigadas e irrigadas diariamente. As avaliações foram realizadas a cada três dias até o ponto de murcha permanente, ou no máximo por 30 dias após a suspensão da irrigação. Concomitantemente, as plantas em diferentes condições hídricas (a partir dos 30 dias) foram irrigadas e avaliadas 24 e 48 horas após a aplicação de água. O tratamento não irrigado promoveu uma redução significativa no potencial hídrico, enquanto a reirrigação promoveu uma recuperação parcial no potencial hídrico. Houve um aumento significativo nos teores de açúcares solúveis totais e redutores, nas folhas e raízes das plantas do tratamento não irrigado. Nas plantas que tiveram uma rerrigação se comportou como as plantas que permaneceram sem irrigação, contudo, menores teores de açúcares foram detectadas nas mesmas. As folhas e raízes das plantas sem irrigação também apresentaram significativa redução nos teores de amido. A atividade da sintase da sacarose e invertase, neutra e ácida, aumentou em função do estresse hídrico.*

Termos para indexação: *Coffea arabica*, *seca, sintase de sacarose, invertases, açúcares solúveis totais.*

1 INTRODUCTION

Coffee is one of the most important crops in agriculture around the world. There are around 100 coffee species described in the Coffea genus (DAVIS et al., 2006). *Coffea arabica* and *Coffea canephora* Pierre ex. A. Froehner are the most important coffee species, accounting for 62% and 38% of the global market, respectively (DIAS et al., 2007). Brazil is the biggest coffee producer in the world. It produced 48 million bags of coffee in 2010.

Climate changes challenge worldwide plant production (WANG; VINOCUR; ALTMAN, 2003; WHITE; MCMASTER; EDMEADES, 2004), especially in arid and semiarid regions where, in addition to drought, there are problems with soil salinity. Furthermore, in addition to crop growth and yield, crop quality is also expected to be affected by global climatic changes (DAMATTA et al., 2010).

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Plants have developed biochemical responses to adjust their metabolism according to environmental changes. Responses may be complex and may occur at the morphological, physiological and molecular level; however, they will depend on the plant genotype, stress intensity and duration, plant growth stage and the nature of the stress. The solutes intracellular storage and distribution in response to water stress conditions and salinity are important mechanisms to tackle water stress (TURNER, 1986). The osmotic adjustment is considered one of the most efficient mechanisms to maintain the cell turgent (CHAVES, 1991). It controls mainly the stomatal opening and photosynthesis under low soil water potential.

Several investigations have been conducted to study tolerance to water stress in coffee. Most of them have been conducted in C. canephora (LIMA et al., 2002; PINHEIRO et al., 2005; PRAXEDES et al., 2006; SILVA et al., 2010) and C. arabica (BATISTA et al., 2010; DIAS et al., 2007). The Siriema cultivar has an ability to maintain its leaf area and vigour under drought conditions (DIAS et al., 2007). To date, drought tolerant coffee plants have been catalogued empirically because there is a lack of knowledge about their physiological responses to water stress (DAMATTA; RAMALHO, 2006).

The objective of this research was to study the effect of drought conditions on the leaf water potential, carbohydrate concentrations, sucrose synthase and invertases activity in Siriema coffee seedlings. It was also aimed at evaluating Siriema's capacity to recover from water stress conditions.

2 MATERIALS AND METHODS

Plant material and growing conditions

The experiment was conducted in a greenhouse with a 50% light reduction cover. This greenhouse was located in the Plant Physiology experimental area of the Federal University of Lavras, Minas Gerais, Brazil. Six-month-old Coffea arabica cv. Siriema seedlings were used for the experiments. The plants were grown in plastic bags (three liter capacity).

The plants were irrigated daily, and the water potential was kept at field capacity for three weeks. When the treatments started, one group of seedlings was continuously irrigated (control) while another group was not irrigated for a 30 day period. In addition, plants subjected to different drought conditions (from 3 to 30 days of water stress/withholding) were re-irrigated at each time point and evaluated 24 and 48 hours later. Evaluations were done every 3 days for a 30 day period. The leaf water potential was evaluated with a pressure chamber (Soil moisture – Mod. 3005), early in the morning, in the fourth pair of expanded leaves (in three seedlings per treatment). At the evaluation time, leaf and root samples were frozen in liquid nitrogen and subsequently stored at -80 ºC until their biochemical analysis took place. All the plants were grown in a completely randomized design, with three repetitions per evaluation period. The biological repetition consisted of a single plant.

Extraction and quantification of total soluble sugars, reducing sugars, and starch

The extraction and quantification of the total soluble sugars, reducing sugars and starch was performed in coffee leaves and roots according to Silva et al. (2003). The reducing sugars quantification was done using the Miller (1959) protocol. For the total soluble sugars and starch quantification, the procedure conducted by Yemm and Coccking (1954) was used.

Extraction and quantification of the sucrose synthase and invertases activity

The extraction and quantification of the sucrose synthase enzyme (Susy) was performed in coffee leaves and roots according to the Cazetta, Seebauer and Below (1999) methodology. The dinitrosalicilic acid (DNS) method was used for the reducing sugars quantification (MILLER, 1959); the enzyme activity was measured after 40 minutes of sample incubation. The soluble invertases (cytosolic neutral invertase and vacuolar acid invertase) extraction and quantification was conducted with the Zeng et al. (1999) procedure and the insoluble invertase (cell wall acid invertase) activity was quantified with the Cazetta et al. (1999) protocol.

For the cytosolic neutral invertase and vacuolar acid invertase 0.2 g of fresh tissue was used for the extraction (from leaves and roots). This tissue was homogenized in 2 ml of extraction buffer: 200 mM HEPES (pH 7.5), 1 mM PMSF, 5 mM $MgCl_2$, 1 mM DDT and 50 mM ascorbic acid. Next, the samples were centrifuged at 18,000 × g for 20 minutes at 4 ºC. The supernatant was collected for the analysis of the soluble invertases

and the precipitate was resuspended in 2 ml of 200 mM sodium acetate buffer (pH 4.5), 1 mM PMSF, 5 mM $MgCl_2$, 1 mM DTT, 50 mM ascorbic acid and 1 M NaCl to extract the cell wall acid invertase. Finally the sample was centrifuged 18,000 × g for 20 minutes at 4 ºC and the supernatant was stored for the enzyme analysis. The 100 mM potassium phosphate buffer (pH 7.5) was used to measure the cytosolic neutral invertase activity. The 200 mM sodium acetate buffer (pH 4.5) with 5 mM $MgCl_2$ and 200 mM sucrose was used for the vacuolar acid invertase and cell wall acid invertase activity assays. The incubation was performed in a water bath for 40 minutes at 30 ºC. After that, 150 µl of 1 N NaOH solution was added to the cytosolic neutral invertase assay and 300 µl was added to the vacuolar acid invertase and cell wall acid invertase assays in order to stop the enzymatic reaction. To quantify the products, a DNS method for reducing or sugars (MILLER, 1959) was used. The enzymatic activity was quantified after 40 minutes incubation.

Data analysis

Statistical analyses were performed with the InfoStat/Profesional version 1.1 software. Pearson correlations were carried out once the data were tested for normality with a Shapiro-Wilks test. Transformation with a log (10) was used when data were not a normal function. When it was not possible to normalize the data, a Spearman correlation coefficient was used.

3 RESULTS AND DISCUSSION

Leaf water potential

The leaf water potential in Siriema coffee seedlings growing under drought conditions was evaluated. Plants cultivated under this condition decreased their water potential, reaching -3.5 MPa after 30 days (Figure 1). The control, on the other hand, presented no changes in leaf water potential during the experiment. To analyze whether the coffee seedlings were able to recover their water potential, plants were re-irrigated and evaluated 24 and 48 hours after. The results showed that coffee plants recovered their water potential even after being subjected to 24 days of drought conditions. A higher recovery was observed in the plants evaluated 48 hours after re-irrigation than the plants evaluated 24 hours later, even though, their water potentials were similar to the control values in the first 10 days of experiment. After 27 days, the leaf water potential of the Siriema cultivar was about –3.5MPa. At day 30, it was not possible to evaluate the effect of 24 and 48 hour re-irrigation treatments because the leaves had already been abscised from the plants (Figure 1).

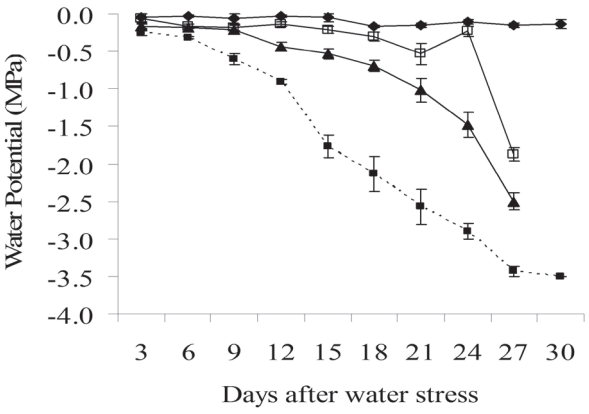

FIGURE 1 – Leaf water potential evaluated in seedlings of the Siriema coffee cultivar under drought conditions. Plants were grown under drought conditions for a 30 days period. Four treatments were tested: Irrigated plants (–♦–), non irrigated plants (--■--), plants that were re-irrigated after a certain water stress period and evaluated 24 (–▲–) and 48 hours (–□–) post-irrigation. Mean values are presented; dashes shows the standard errors.

Although there are variations throughout the day, the leaf water potential describes the plant water status level and consequently has been widely used to study water relations in plants (WESTGATE, 1994). When coffee plants are growing in the field, they can survive water potentials as low as -4.0MPa during the dry season (MARRACCINI et al., 2011). In nature, water stress happens slowly and gradually, allowing the plants to adjust to this unfavourable growing condition. In addition to that, the root system can reach deep in the soil and the leaf area index is higher. On the other hand, under the experimental conditions applied on this work, where the stress was faster and stronger then in nature, the Siriema plants still could survive and recover until about -3,0 MPa after 24 days under drought conditions.

Carbohydrate concentrations

The total soluble sugars (TSS) increased as a drought response in coffee. The TSS remained constant in the control treatment during all the water stress periods, both in leaves and roots. In the drought treatments, on the other hand, the TSS content in leaves increased after day 9 (Figure 2).

The highest TSS values were obtained at days 24 and 27, and the water potential was also at its lowest during those days (Figure 1). The roots showed a similar trend, but their total soluble sugars (TSS) increased after 3 days of water stress (Figure 2). The highest TSS values were observed on day 24 (21.97 mg / g FW^{-1}).

Osmotic adjustment is a mechanism that happens in plants under water stress conditions, and it is characterized by the accumulation of intracellular osmotically active solutes (CHAVES, 1991). Some carbohydrates can function as compatible solutes maintaining the hydric potential, and, in addition, they protect

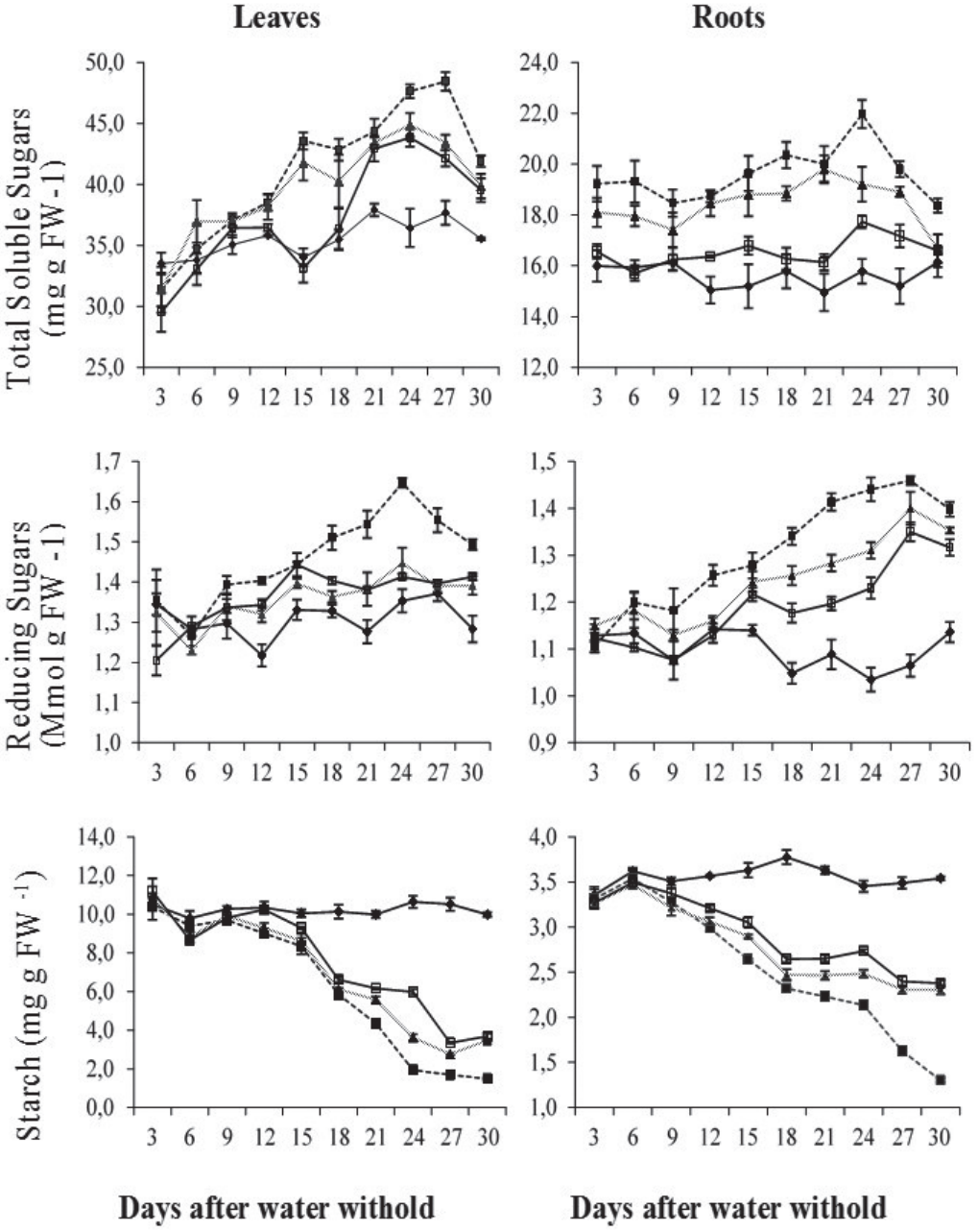

FIGURE 2 – Carbohydrate concentrations in leaves and roots of seedling of the Siriema coffee cultivar under drought conditions. Plants were grown under drought conditions for a 30 day period. Four treatments were tested: Irrigated plants (–♦–), non irrigated plants (--■--), plants that were re-irrigated and evaluated 24 (–▲–) and 48 hours (–□–) after a certain water stress period. Mean values are presented, dashes shows the standard errors. FW means fresh weight.

cells from dehydration by forming vitreous structures (NEPOMUCENO et al., 2001). Osmotic adjustment allows the cells to grow under inhibiting conditions. Changes in solute concentration also regulate stomatal opening and photosynthesis (TURNER, 1996).

Reducing sugars increase under drought conditions in coffee leaves and roots. While the irrigated plants maintained a constant concentration of reducing sugars in the leaves, the plants subjected to the non-irrigated treatment increased their concentration of reducing sugars after day 9 (Figure 2). On day 24 the plants reached their highest concentration (1.65 Mmol / g · FW^{-1}), but the levels of reducing sugars decreased after this day. The same trend was observed in roots (Figure 2), which means that reducing sugars increased under water stress conditions to trigger oxi-reduction reactions. This increase could happen as a result of invertases activity to decrease the water potential if the hexoses released by this enzyme contribute to the osmotical adjustment and prevent additional water-stress-induced cell damage (VALLIYODAN; NGUYEN, 2006). In the Siriema cultivar the opposite changes in carbohydrate levels were found in the drought-stressed plants that were re-irrigated, in which condition the osmotical adjustment is no longer required.

Starch is degraded as a drought response in the leaves and roots of the Siriema cultivar. Under drought conditions, the starch content decreases after 12 days of water stress and reaches values as low as 1.48 mg / FW · g^{-1} (in leaves) and 1.30 mg / FW · g^{-1} (in roots) after 30 days of drought (Figure 2). Re-irrigation increases the starch content (3.69 mg / FW · g^{-1} at day 30). This results shows that a coffee plant's starch reserves are hydrolyzed to overcome drought conditions. Similar results have been observed before in coffee (PRAXEDES et al., 2006).

Sucrose synthase and invertases activity

Sucrose is the main compound exported from leaves to other organs (roots, reproductive organs, shoots, stems) for growth or storage. Its hydrolysis releases hexoses for anabolic or catabolic processes and also to increase reducing sugars for osmotic adjustment. Invertases cleave this process in a higher extend than sucrose synthase (SUSY) (KINGSTON-SMITH; WALKER; POLLOCK, 1999). Even though invertases and SUSY both target sucrose, the activity of these enzymes during growth and plant development is, thus these enzymes become relevant under plant stress conditions, not only to osmotically adjust the cells, but also in order to process such cell wall synthesis. The SUSY activity increased in the leaves of the Siriema coffee cultivar under drought conditions (after 9 days, Figure 3); similar results were observed in the roots (Figure 3). SUSY is mainly involved in cell wall and starch synthesis (WINTER; HUBER, 2000); however, it may also be related to sucrose synthesis since starch content decreases under drought conditions.

In *Coffea canephora*, water potentials lower than 3.0 MPa increase the sugar content, hexose concentration, and acid invertase activity (PRAXEDES et al., 2006). A similar increase in the invertases activity was observed in the Siriema cultivar under drought conditions. The cytosolic neutral invertase (CNI) activity increased in leaves during days 15, 18, and 21 (Figure 3). In roots, on the other hand, the highest CNI activity was detected on day 15 and its activity decreased after this day (Figure 3). Leaf vacuolar acid invertase (VAI) activity also increased under drought conditions (Figure 3); conversely, root VAI activity decreased after day 21 (Figure 3). A similar trend was observed in the plants subjected to the re-irrigation treatments. Their cell wall acid invertase (CWAI) activity increased in leaves under water stress conditions (Figure 3); but CWAI activity decreased after day 24 when it was evaluated in roots (Figure 3). VAI controls the primary sucrose degradation pathway in tissues undergoing expansion (WINTER; HUBER, 2000) and mature tissues, and it also contributes to the hexose flux across the tonoplast that enables hexoses to enter into cytoplasmic metabolism.

Correlations among carbohydrate concentrations and enzyme activities

Differential correlations were estimated among the treatments. Transpiration is positively correlated with the total soluble sugars and with the reducing sugars under drought conditions; opposite correlations were estimated in the re-irrigated plants (data not shown). In addition to that, the invertases activity is positively correlated with stomatal resistance under re-irrigated and irrigated conditions; Moreover, stomatal resistance is higher under water stress conditions than under re-irrigated conditions (data not shown). Also there is a positive correlation between transpiration, that increases in re-irrigated plants (data not shown), and the starch content. Differential correlations

between various carbohydrates and different enzyme activities show the complexities of water stress and water stress recovery metabolism and demonstrate that these physiological responses involve more than one compound and more than one enzyme (Figure 4).

The previous results show how carbohydrate concentrations and enzyme activities (invertases) play a role to adjust the metabolism under water stress conditions. Consequently, they should be considered when breeding or genetic engineering drought tolerant coffee plants.

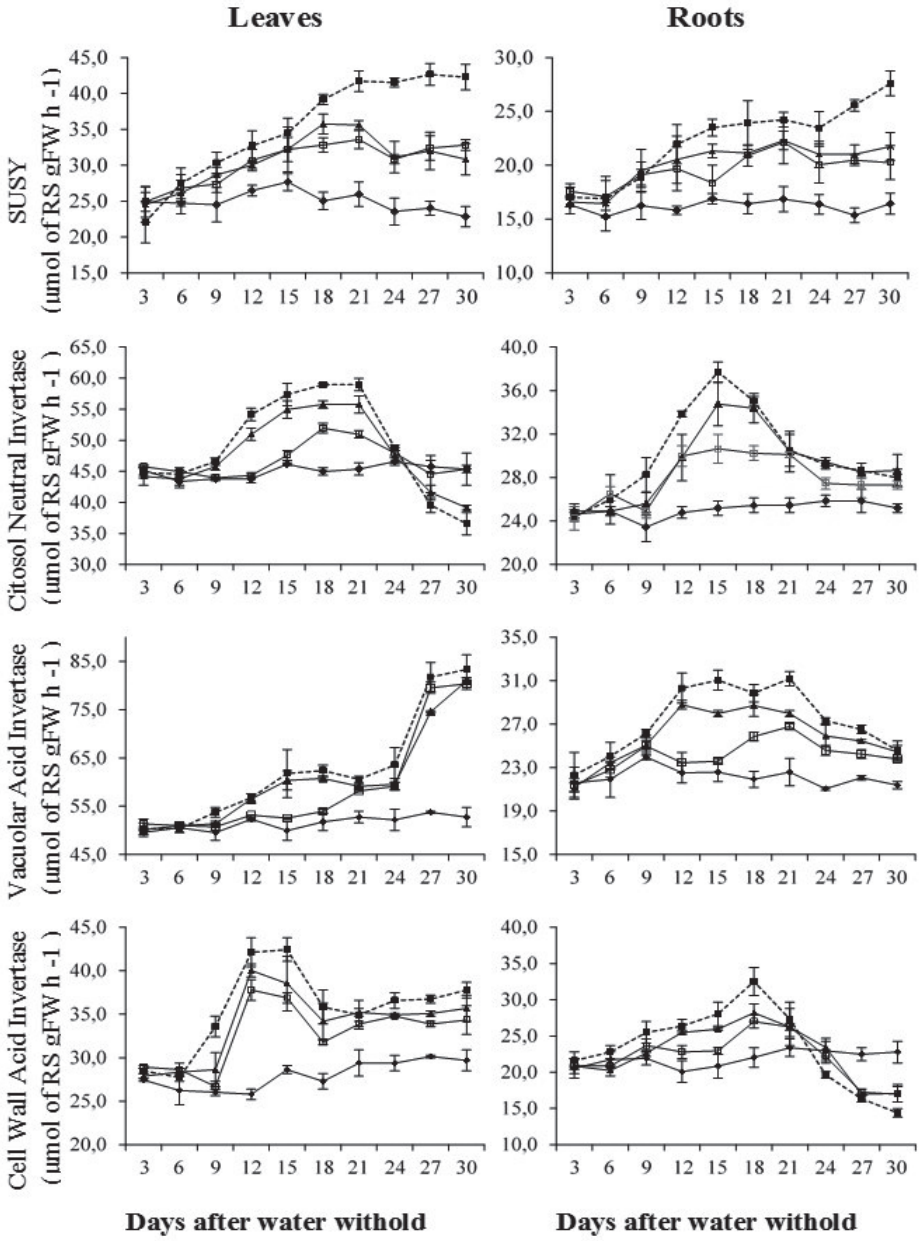

FIGURE 3 – Enzyme activity related to carbohydrate metabolism in leaves and roots of seedlings of the Siriema coffee cultivar grown under drought conditions. Plants were grown under drought conditions for a 30 day period. Four treatments were tested: Irrigated plants (–♦–), non irrigated plants (--■--), plants that were re-irrigated and evaluated 24 (–▲–) and 48 hours (–□–) after a certain water stress period. Mean values are presented, dashes shows the standard errors. RS means Reducing sugars, h means hour and FW means fresh weight.

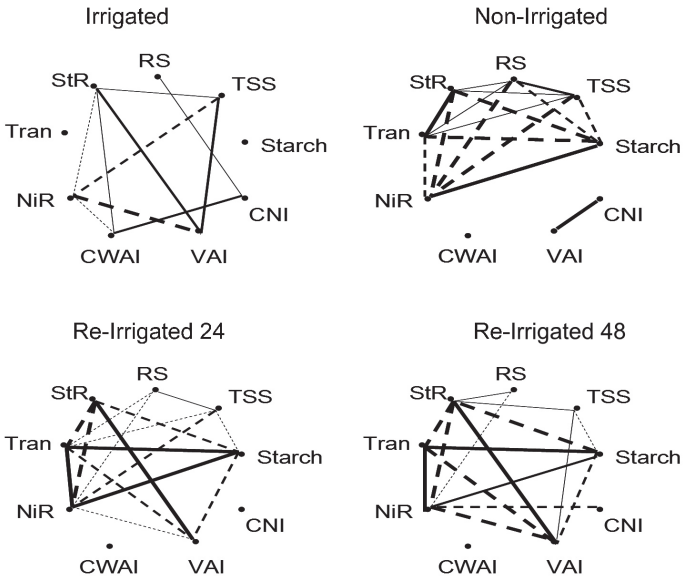

FIGURE 4 – Correlations between reducing sugars (RS), total soluble sugars (TSS), starch, cytosolic neutral invertase (CNI), vacuolar acid invertase (VAI), cell wall acid invertase (CWAI), nitrate reductase (NiR), transpiration (Tran) and stomatal resistance (StR) in leaves of seedlings of the Siriema coffee cultivar that were growing under irrigated and non-irrigated conditions. In addition, data are presented from plants that were grown on drought conditions and evaluated 24 hours (Re-irrigated 24) or 48 hours (Re-irrigated 48) after re-irrigation. Filled lines (—) mean positive correlations; dotted lines (---) are negative correlations. The thinnest lines (ı) means a correlation coefficient between 0.65-0.80, the intermediate line between 0.8 – 0.9, and the thickest line (▌) between 0.9-1.0. The significance level for plotting the correlations was $P < 0.05$.

4 CONCLUSIONS

Siriema coffee seedlings can withstand drought conditions for a 30 day period. These plants are able to recover their water potential when re-irrigation is performed following 24 days of drought. Under these same conditions, specific enzymatic activities increase to cleave storage carbohydrates in leaves and roots. A negative correlation between starch and reducing and total soluble sugars was observed. In addition, the invertases and sucrose synthase activities changed under drought conditions and consequently these enzymes are important to be considered for further studies.

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