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Germination and biochemical changes in West Indian gherkin seeds under water stress at different temperatures[1]

Germinação e alterações bioquímicas em sementes de maxixe sob estresse hídrico em diferentes temperaturas

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ABSTRACT - Information on the effects of climate conditions on the survival of species grown in semi-arid regions is essential and at the same time scarce, especially regarding rustic species such as West Indian gherkin (*Cucumis anguria* L.). Thus, this study aimed to evaluate germination, growth and biochemical alterations in *C. anguria* seeds under water stress and different temperature regimes. The experimental design was completely randomized, in 5 x 5 factorial scheme, composed of five osmotic potentials (0.0; -0.1; -0.2; -0.3 and -0.4 MPa) and four temperatures (20; 25; 30 °C and alternating temperatures of 20-30 ºC), with each treatment composed of four replicates of 50 seeds. The seeds were sown on paper towel (Germitest®) and germinated in *Biochemical Oxygen Demand* (B.O.D.) germinators, under 8-h photoperiod. Germination percentage, germination speed index, mean time of germination, seedling length and contents of chlorophylls, carotenoids, proline, free amino acids and free sugars were determined along the experiment. Decrease in osmotic potential reduced germination, growth and contents of chloroplast pigments of *C. anguria* seedlings, but was more drastic at levels lower than -0.2 MPa. Low temperatures intensify the effects of water stress on the germination of *C. anguria* seeds. The synthesis of protecting osmolytes increased in *C. anguria* seedlings but these components did not promote efficient osmotic adjustment in their initial development stage.

Key words: *Cucumis anguria*. Cucurbitaceae. Water deficit. Vigor.

RESUMO - As informações sobre os efeitos das condições climáticas na sobrevivência de espécies cultivadas em regiões semiáridas são imprescindíveis e, ainda, escassas, principalmente em se tratando de espécies rústicas como o maxixe (*Cucumis anguria* L.). Com isso, objetivou-se avaliar a germinação, crescimento e alterações bioquímicas em sementes de *C. anguria* sob estresse hídrico em diferentes regimes de temperaturas. O delineamento experimental utilizado foi o inteiramente casualizado, em esquema fatorial 5 x 5, constituído de cinco potenciais osmóticos (0,0; -0,1; -0,2; -0,3; -0,4 MPa) e quatro temperaturas (20; 25; 30 °C e alternada 20-30 °C), com cada tratamento composto por quatro repetições de 50 sementes. As sementes foram semeadas em papel toalha (Germitest®) e colocadas para germinar em germinadores do tipo *Biochemical Oxygen Demand* (B.O.D.), com fotoperíodo de oito horas de luz. Durante a condução do experimento, determinou-se a percentagem de germinação, índice de velocidade de germinação, tempo médio de germinação, comprimento das plântulas, teores de clorofilas e carotenoides, teores de prolina, aminoácidos livres e açúcares livres. A redução do potencial osmótico diminuiu a germinação, o crescimento e o teor de pigmentos cloroplastídicos das plântulas de *C. anguria*, porém sendo mais drástica em níveis inferiores a -0,2 MPa. As baixas temperaturas potencializaram os efeitos do estresse hídrico na germinação de sementes de *C. anguria*. Ocorreu aumento da síntese de osmólitos protetores em plântulas de *C. anguria*, porém estes não promoveram ajuste osmótico eficiente na fase inicial de desenvolvimento da plântula.

Palavras-chave: *Cucumis anguria*. Cucurbitaceae. Déficit hídrico. Vigor.

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INTRODUCTION

Cucumis anguria L., from the Cucurbitaceae family, is popularly known in Brazil as ‘maxixe’ and is an annual, rustic plant normally grown in small scale (FILGUEIRA, 2008). Due to its rusticity, it has been considered as an option for family farming in the North and Northeast regions of Brazil (OLIVEIRA *et al*., 2013). Despite its high production potential, there are still few studies to allow the expansion of this crop, and there is no technology to make it attractive to the producers (ALVES *et al*., 2014).

Information on the effects of climate conditions on the survival of species grown in semi-arid regions are essential and, at the same time, scarce. Therefore, it is extremely important to know the factors which limit the initial development of the species, especially abiotic factors such as water and thermal deficits, which affect seed germination and the initial development of plants (CARVALHO; NAKAGAWA, 2012; MARCOS-FILHO, 2015). Studies related to the germinative response of seeds to the condition of artificial stresses are important for ecophysiology and constitute tools which allow for the evaluation of limits and tolerance of survival and adaptation of these species to the conditions of natural stresses (GUEDES *et al*., 2013).

For a viable seed to be able to germinate, several factors are necessary such as water supply in sufficient amount, temperature, substrate and adequate composition of gases (CARVALHO; NAKAGAWA, 2012). Presence of an adequate level of hydration which favors the reactivation of metabolic processes is crucial for germination and, consequently, for embryo axis growth (MARCOS-FILHO, 2015). Water stress usually acts to reduce seed germination percentage and speed, and for each species there is a value of water potential in the soil below which germination does not occur (LOPES; MACEDO, 2008).

One of the techniques employed in the laboratory to simulate conditions of low moisture in the substrate has been the use of aqueous solutions with different osmotic potentials (GUEDES *et al*., 2013; MARCOS-FILHO, 2015). Currently, polyethylene glycol (PEG 6000) has been successfully used in research studies to simulate the effects of water deficit on horticultural species, since it promotes slow and controlled imbibition of seeds (ALVES *et al*., 2014; LIMA; MARCOS-FILHO, 2010; SILVA *et al*., 2014).

Another abiotic factor which influences the process of germination and, consequently, seedling development is temperature. Germination occurs within relatively wide limits of temperature, and the extremes depend mainly on the species and its genetic traits (MATHEUS; LOPES, 2009). Gradual reduction of temperature causes a sharp decrease in germination speed and may cause reduction in seedling growth, even when the temperature returns to favorable levels (MARCOS-FILHO, 2015). Optimal temperature ensures the best combination between germination percentage and speed (NASCIMENTO, 2013).

However, although there is research involving the physiological behavior of seeds at different temperatures, none of the studies found in the literature seek to evaluate the effect of water stress, as well as the combination of abiotic factors, such as thermal stress x water stress, conditions to which the species is subjected in nature.

Thus, the objective was to evaluate the germination, growth and biochemical alterations in *C. anguria* seedlings under water stress and different temperature regimes.

MATERIAL AND METHODS

The experiment was conducted at the Seed Analysis Laboratory (LAS) of the Center of Agrarian Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró - RN, Brazil (5°11’S, 37°20’ W and altitude of 18 m).

The experimental design was completely randomized, in factorial scheme with five osmotic potentials and four temperatures, and each treatment comprised four replicates of 50 seeds.

Seeds of *C. anguria*, cv. ‘Do Norte’, were subjected to water stress, using polyethylene glycol (PEG 6000) solutions prepared according to the values indicated by Villela, Doni Filho and Siqueira (1991) to simulate the pre-established osmotic levels (0.0; -0.1; -0.2; -0.3; -0.4 MPa), besides the control with distilled water. Seeds were sown on paper towel (Germitest®) sheets moistened with PEG 6000 solutions, using an initial volume equivalent to 2.5 times the paper towel mass. The repetitions of each treatment were placed in transparent plastic bags, to avoid water loss through evaporation during the test.

Germination test was conducted in *Biochemical Oxygen Demand* (B.O.D.) germinators, regulated at constant temperatures of 20, 25, 30 °C and alternating temperatures of 20-30 ºC, under 8-h photoperiod. Counts were made daily until the 11th day after sowing, considering as germinated the seeds which produced primary root and healthy seedling shoots (BRASIL, 2009), and the results were expressed in percentage. Germination speed index was determined along with the germination test, by evaluating the seedlings every day from the beginning of germination until the 11th day after sowing (MAGUIRE, 1962). Mean time of germination was determined based on the number of seeds germinated every day, and the results were expressed in days after sowing. At the end of the germination test, all normal seedlings were measured for length using a ruler graduated in millimeters.

Contents of chlorophylls *a* and *b* and carotenoids were determined by chlorophyll extraction in acetone (80%) and quantification by spectrophotometry. Sample absorbance values were recorded in spectrophotometer at 470, 646.8 and 663.2 nm, and the contents of chlorophylls and carotenoids (g pigment kg^{-1} DM) were obtained according to Lichthenthaler (1987), using the following equations: 1- chlorophyll *a*= 12.25 ABS663.2 – 2.79 ABS646.8; 2- chlorophyll *b*= 21.50 ABS646.8 – 5.10 ABS663.2; 3- total carotenoids = (1000 ABS470 – 1.82 chlorophyll *a* – 85.02 chlorophyll *b*)/198.

At the end of the experiment, samples of seedling shoot and root fresh mass were collected in the different treatments to determine total soluble sugars, total free amino acids and proline. The content of total soluble sugars was determined by the anthrone method (YEMM; WILLIS, 1954), with results expressed in μmol of GLU g^{-1} of fresh matter. Amino acids contents were quantified by measuring the absorbance at 570 nm and applying the acid ninhydrin method (YEMM; COCKING, 1955), using glycine as standard substance, and the results were expressed in μmol TFAA g^{-1} of fresh matter. Proline was quantified by following the methodology described by Bates, Waldren and Teare (1973), and the results were expressed in μmol PRO g^{-1} of fresh matter.

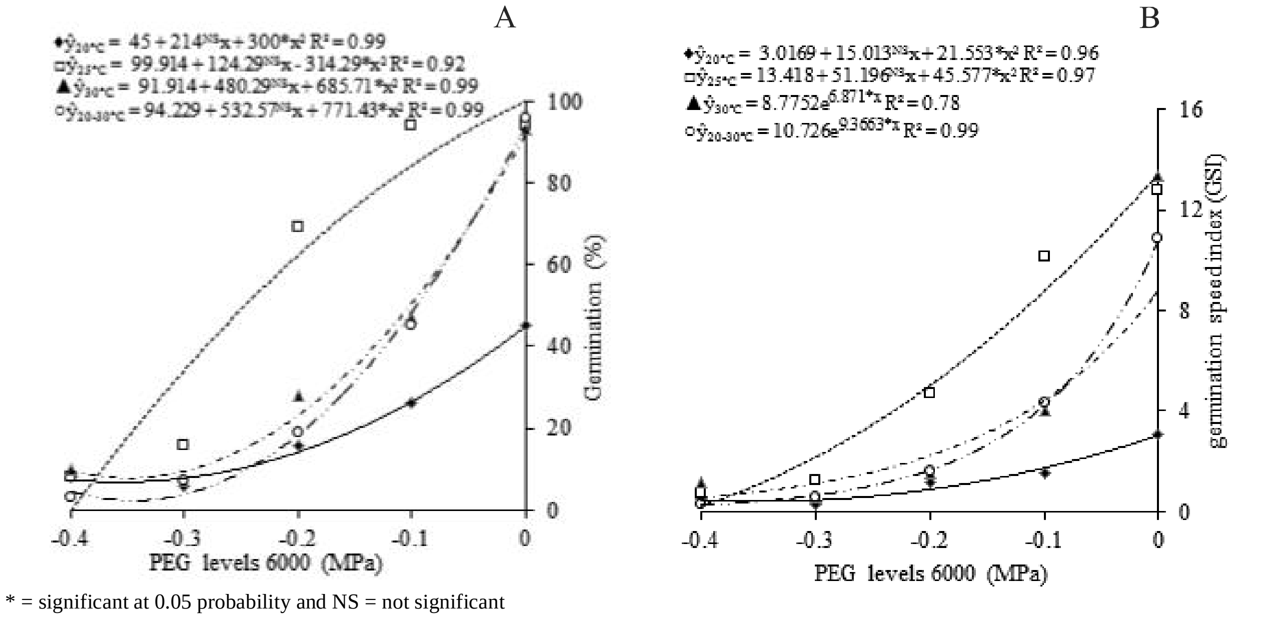
The results were subjected to analysis of variance by F test at 0.05 probability level and, in case of significance, the data were subjected to regression analysis using the statistical program SISVAR® (FERREIRA, 2011).

RESULTS AND DISCUSSION

The interaction between osmotic potentials and temperatures significantly influenced ($p<0.05$) the variables germination, germination speed index, chlorophyll *a*, chlorophyll *b*, carotenoids, proline, total sugars and total amino acids. Mean time of germination and seedling total length were significantly affected ($p<0.05$) by the each of the studied factors alone.

Highest percentages of germination (96%, 94% and 93%) were obtained for *C. anguria* seeds subjected to temperatures of 20-30, 25 and 30 °C at the level zero (control). As the osmotic potentials decreased, there was a drastic reduction in the germination percentage of seeds at all temperatures tested, except for those germinated at 25 ºC, which maintained favorable percentages up to a osmotic potential of -0.2 MPa, evidencing that under these environmental conditions *C. anguria* tolerates more water stress than at other temperatures (Figure 1A). This fact may be related to the absence of thermal stress because this temperature is indicated for germination tests with this species (BRASIL, 2009).

Figure 1 - Germination (A) and germination speed index (GSI) (B) of West Indian gherkin (*Cucumis anguria* L.) seeds subjected to different temperatures and water stress levels



* = significant at 0.05 probability and NS = not significant

Highest reductions in germination percentage were observed at 20 ºC and alternating temperatures of 20-30 ºC, reaching 3% of germination at 20-30 ºC under a potential of -0.4 MPa (Figure 1A). A possible explanation for this result is related to the fact that PEG 6000 has high viscosity and molecular weight, which retard the rate of tissue hydration and oxygen diffusion, requiring longer time for the reorganization of membranes and development of metabolic processes (ANTUNES *et al*., 2011). Furthermore, there is also a certain sensitivity of cucurbits to the stress at low temperatures.

Reduction of germination due to increased water deficit was also observed by Medeiros *et al*. (2015) in sesame (*Sesamum indicum* L.) seeds, whose physiological performance was affected by water stress, with significant reductions in germination and vigor; however, the temperature of 30 °C favored the germinative performance of this species. Silva *et al*. (2016) found that reduction in the osmotic potentials from -0.2 MPa negatively affected the germination and vigor of 'paineira' (*Chorisia glaziovii* Kuntze) seeds, especially at temperature of 20 °C. On the other hand, sunflower (*Helianthus annuus* L.) seeds showed resistance to water stress and their maximum tolerance limit was -0.4 MPa (CARNEIRO *et al*., 2011).

As observed for germination, the germination speed index (GSI) of *C. anguria* seeds was also affected by the osmotic potentials at the different temperatures, and the highest indices were observed in the control treatment at temperatures of 25 and 30 ºC (Figure 1B). Lowest values of GSI were found at temperature of 20 °C for all osmotic potentials studied, so that, in addition to a slower germination process, the seeds were less tolerant to water stress under this temperature condition (Figure 1B). According to Carvalho and Nakagawa (2012), certain limits of temperature, either above or below the optimal limit, tend to reduce germination speed, causing disorganization of membranes and leading to reduction in total germination. In the present study, these reductions tend to be intensified by water stress, which limits seed imbibition, compromising all metabolic processes involved in germination (MARCOS-FILHO, 2015).

Reduction in germination speed, increasing the time between sowing and the beginning of the production of primary root, is probably due to the lower water absorption by seeds, because the increase in osmotic concentration causes reduction in the hydraulic gradient of the substrate-seed system (MARCOS-FILHO, 2015). Similar results were also found by Yamashita and Guimarães (2010) in *Conyza canadensis* and *C. bonariensis* subjected to PEG-induced water stress. These authors observed significant reduction of germination and germination speed already from -0.2 MPa, reaching values lower than 50% of the percentage found in the control treatment (0.0 MPa), when seeds were placed to germinate at a potential of -0.4 MPa.

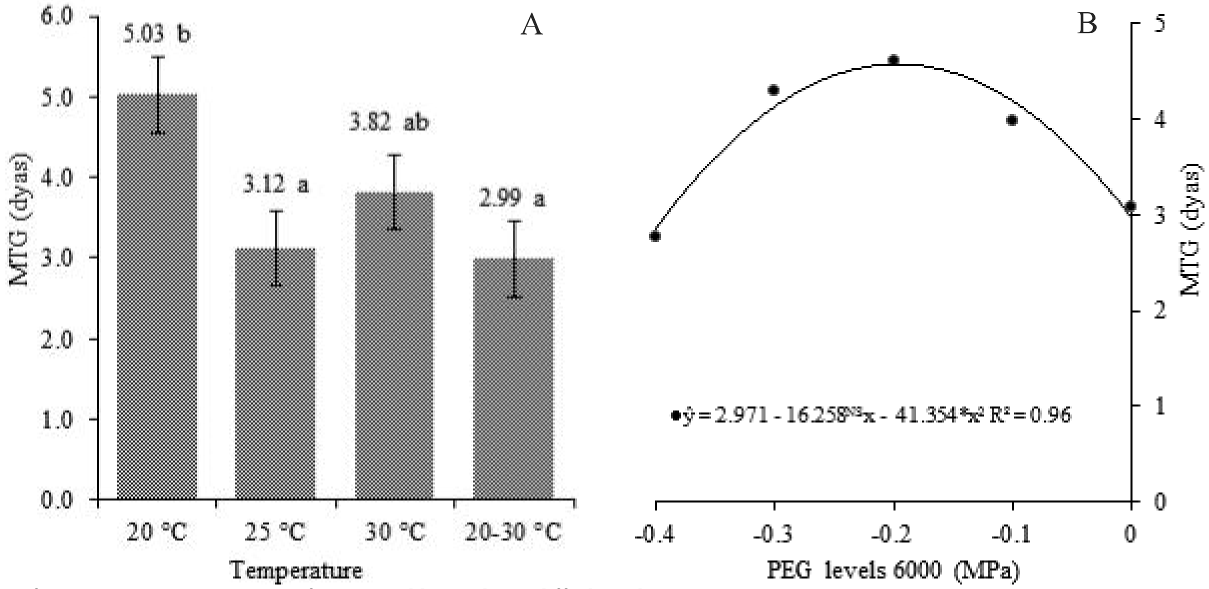
The longest mean time of germination occurred at temperature of 20 °C, at which the germination process occurred more slowly than at the other temperatures studied, indicating that low temperatures retard the germination of *C. anguria* seeds (Figure 2A). Gradual reduction of temperature has effects on the rate of imbibition and mobilization of reserves; in addition, embryo axes subjected to these conditions lose organic substances because their membrane system is damaged, thus causing accentuated reduction in germination speed, consequently increasing the mean time of germination (GORDIN *et al*., 2014; MARCOS-FILHO, 2015).

In relation to the osmotic potentials, there was a quadratic behavior in the mean time of germination of *C. anguria* seeds as the osmotic potential decreased. Longer mean times of germination were found at the osmotic potential of -0.2 MPa, decreasing from this level on (Figure 2B). The shorter mean times of germination observed at the osmotic potentials of -0.3 and -0.4 MPa are not related to the vigor of the seeds, but to the low percentage of germination (< 20%) which occurred in these treatments, given the low values of GSI (Figures 1A and B). Studying the germination of niger (*Guizotia abyssinica*) seeds subjected to water stress simulated with PEG 6000, Gordin *et al*. (2014) observed reductions in the mean time of germination from the potential of -0.4 MPa, which also corroborate the reductions in seed germination and vigor observed in the present study.

As observed in germination, the lowest growth of *C. anguria* seedlings was found at temperature of 20 ºC and alternating temperatures of 20-30 ºC, with reductions of 59% and 50% compared to the growth of seedlings grown from seeds germinated at 25 ºC, respectively (Figure 3A). These results confirm the sensitivity of this species to low temperatures. In relation to the levels of osmotic potential, seedling length showed a linear decreasing behavior, with a reduction of 37% between the lowest level of osmotic potential (-0.4 MPa) and the control (0.0 MPa) (Figure 3B). The results found in the present study agree with those found by Alves *et al*. (2014), who subjected *C. anguria* seeds to water and salt stresses. These authors also observed negative influence of osmotic potential reduction on the germination and vigor of *C. anguria* seeds, with drastic effects at osmotic potentials lower than -0.3 MPa (induced by PEG 6000).

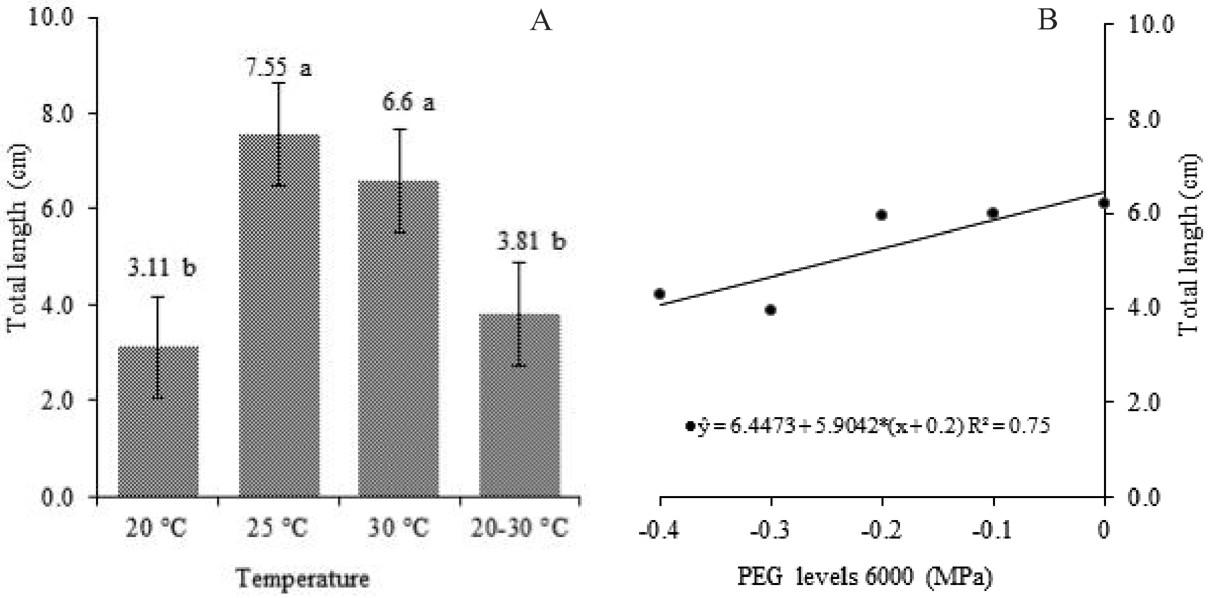
Low water availability, besides limiting imbibition and hence germination, reduces seedling growth due to the decrease in cell growth and expansion (CARVALHO; NAKAGAWA, 2012). Similar results to those found in this study were reported by Silva *et al*. (2014) in Land

Figure 2 - Influence of temperature (A) and water stress levels (B) on the mean time of germination (MTG) of West Indian gherkin (*Cucumis anguria* L.) seeds



* = significant at p <0.05; NS = not significant. Equal letters do not differ by Tukey test (p<0.05)

Figure 3 - Influence of temperature (A) and water stress levels (B) on the total length of West Indian gherkin (*Cucumis anguria* L.) seedlings



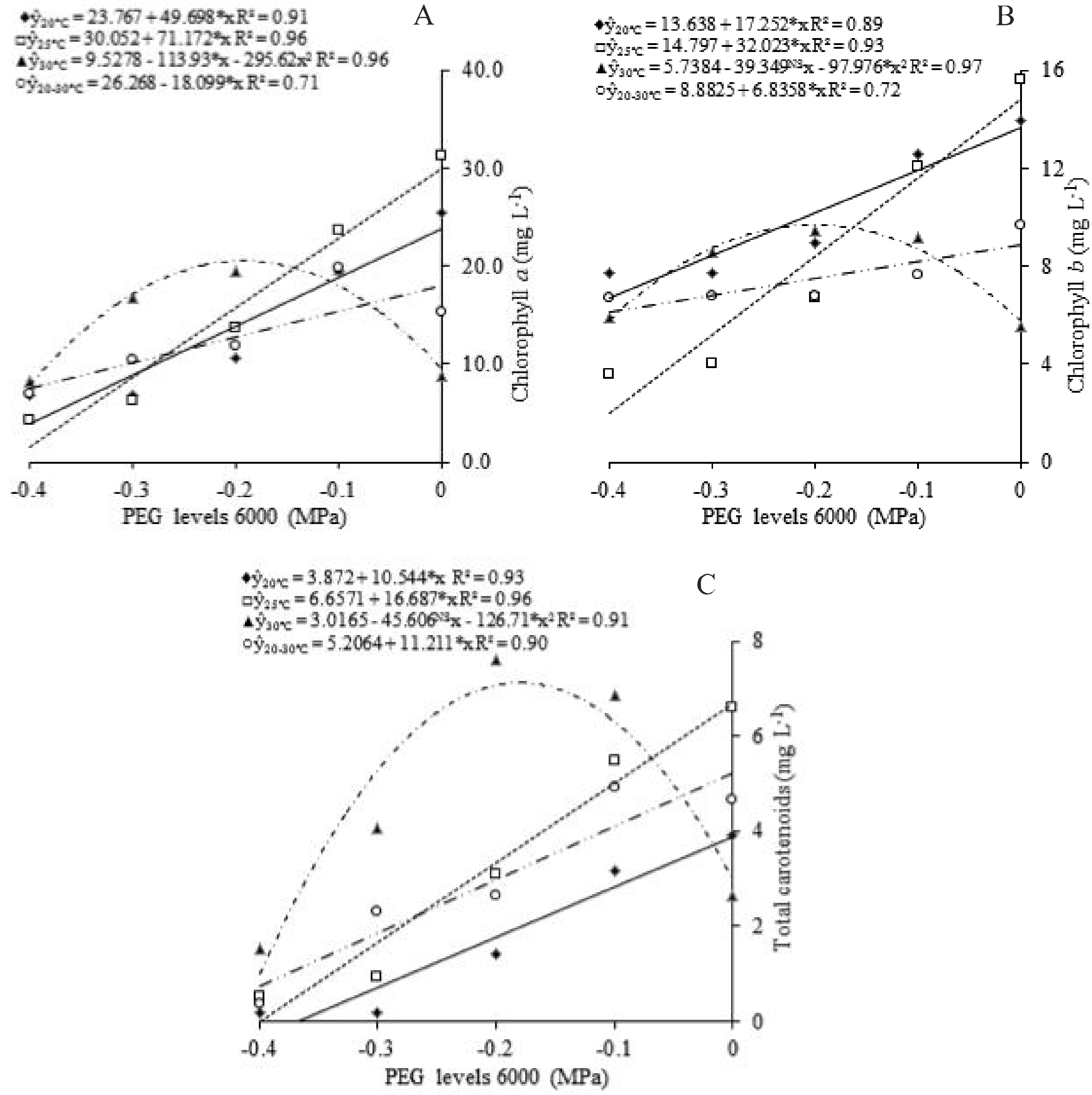
* = significant at p<0.05; NS = not significant. Equal letters do not differ by Tukey test (p<0.05)

cress (*Barbarea verna*) seedlings. These authors observed greater growth of seedlings at temperatures of 25 and 30 ºC and found that the temperature of 20 ºC inhibited seedling growth. For this same temperature, Steiner *et al.* (2010) found restrictions to germination and initial growth of arugula.

Reduction in the osmotic potential negatively influenced the synthesis of chloroplast pigments in *C. anguria* seedlings germinated at temperatures of 20, 25 and alternating temperatures of 20-30 ºC, leading to reductions of 4.96, 7.12 and 1.81 mg L^{-1} for chlorophyll *a*, 1.73, 3.20 and 6.84 mg L^{-1} for chlorophyll *b* and 1.05, 1.67 and 1.12 mg L^{-1} for carotenoids for every -0.1 MPa reduction in the osmotic potential, respectively (Figures 4A, B and C). The chloroplast pigments of seedlings germinated at temperature of 30 ºC showed a quadratic behavior when subjected to the different osmotic potentials, and higher contents of chlorophyll *a*, chlorophyll *b* and total carotenoids were found at potentials of -0.19, -0.20 and -018 MPa, respectively (Figures 4A, B and C). The increase in the contents of chlorophylls and carotenoids in the seedlings at temperature of 30 ºC may be related to their energy status because the stressful condition (water deficit + high temperatures) generates higher expenditure of energy to maintain metabolic activity (TAIZ *et al.*, 2015). This fact causes the seedling to rapidly consume seed reserves, forcing it to obtain energy through photosynthesis earlier than those germinated in the absence of stress.

As the substrate osmotic potential decreased at all temperatures, there was an increase in the synthesis of osmoregulatory solutes (proline, free amino acids and total free sugars) in *C. anguria* seedlings (Figures 5A, B and C). At temperature of 25 ºC, a quadratic behavior was observed in the synthesis of osmoregulatory solutes, and highest contents of proline, free amino acids and free sugars were found at the osmotic potentials of -0.38, -0.33 and -0.29 MPa (Figures 5A, B and C). By comparing the results of germination, growth and concentrations of osmoregulators, at 25 ºC, it can be observed that the synthesis of osmoregulators increases with the increment of water stress, at osmotic potential levels < -0.2 MPa,

Figure 4 - Contents of chlorophyll *a* (A), chlorophyll *b* (B) and total carotenoids (C) in West Indian gherkin (*Cucumis anguria* L.) seeds germinated at different temperatures and water stress levels

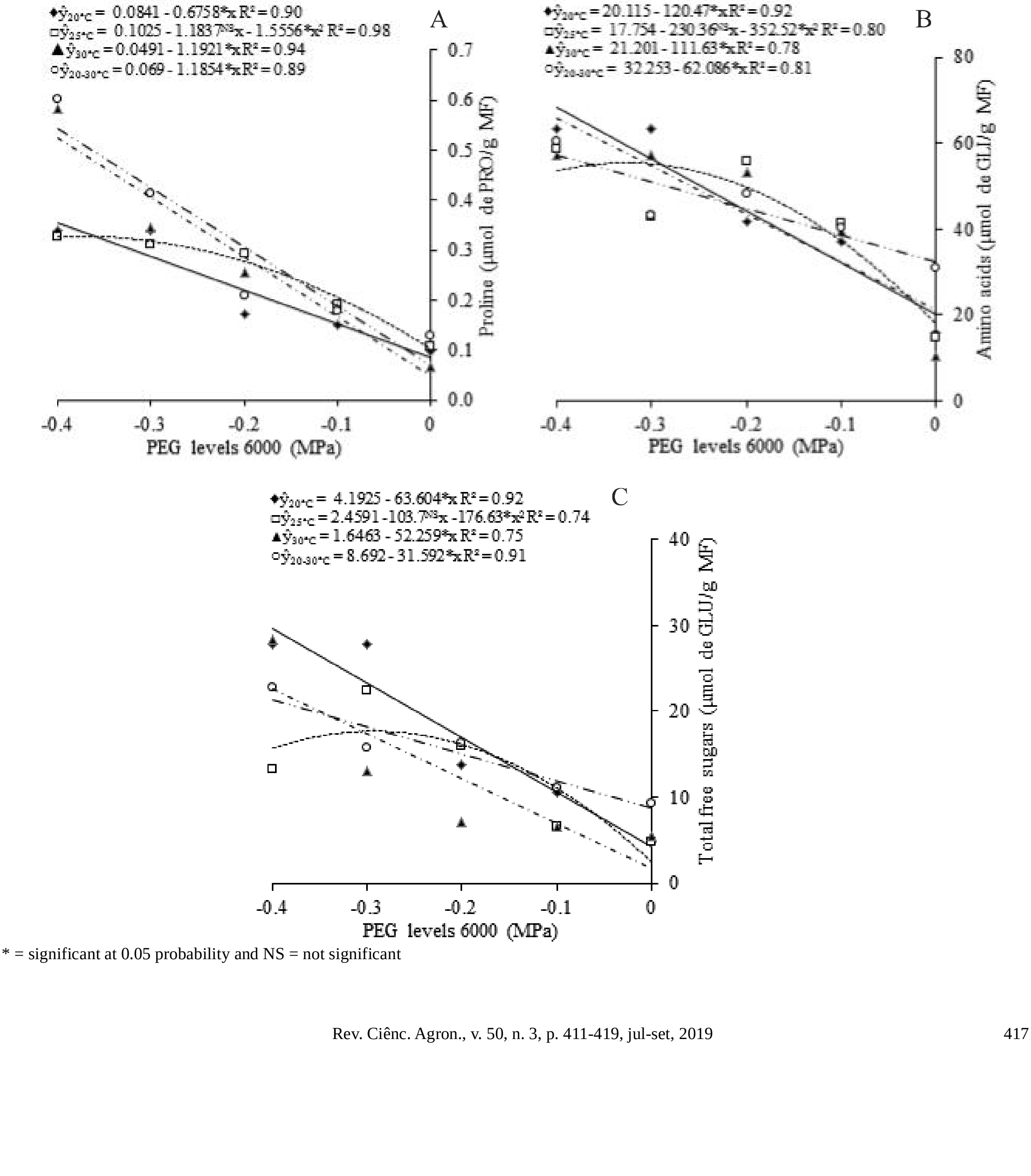


* = significant at 0.05 probability and NS = not significant

evidenced by the good performance observed in germination and growth variables (Figures 1A and 3B). The temperature of 25 ºC is within the optimal range for germination of *C. anguria* seeds and is indicated for their germination test (BRASIL, 2009). Under these conditions, the absence of thermal stress allows the plant to better express its mechanisms of tolerance to water stress, allowing for higher germination and growth, compared to seeds germinated at the other temperatures (Figures 1A and 3A).

Linear increments were observed in the synthesis of proline, free amino acids and free sugars with the reduction in the osmotic potential, in *C. anguria* seedlings germinated at temperatures of 20, 30 ºC and alternating temperatures of 20-30 ºC (Figures 5A, B and C). This result is an indication of the intensity of water stress on the seedlings of this species because the increase in the synthesis of osmoregulatory solutes (osmotic adjustment) is one of the physiological strategies of plants. This strategy aims to reduce their internal water potential to levels lower than those found in the substrate, allowing them to absorb water to perform their vital activities and the osmoregulatory solutes to act as protectors against the oxidative stress, minimizing the degradation of biomolecules (GUPTA; HUANG, 2014; TAIZ *et al*., 2015).

Figure 5 - Contents of proline (A), total free amino acids (B) and total free sugars (C) in West Indian gherkin (*Cucumis anguria* L.) seeds germinated at different temperatures and water stress levels



* = significant at 0.05 probability and NS = not significant

Despite the increase in the synthesis of proline, free amino acids and free sugars, the efforts made to minimize the effects of water stress were not sufficient, given the drastic reductions in germination, growth and contents of chlorophyll and carotenoids observed as the water stress increased (Figures 1, 2, 3 and 4). However, the reductions were more drastic at temperature of 20 ºC and alternating temperatures of 20-30 ºC. At these temperatures, there were lower germination percentages and lower growth up to osmotic potential of -0.3 MPa, compared to the other treatments, denoting that low temperatures have higher influence on the expression of defense mechanisms against water stress in *C. anguria* plants.

CONCLUSIONS

1. Decrease in osmotic potential reduces germination, growth and contents of chloroplast pigments in *C. anguria* seedlings, and such reduction is more drastic at levels lower than -0.2 MPa;
2. Low temperatures intensify the effects of water stress on the germination of *C. anguria* seeds;
3. The synthesis of protecting osmolytes in *C. anguria* seedlings is increased, but such increment does not promote efficient osmotic adjustment in their initial development stage.

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