Thermal stress and physiological changes in watermelon seeds[1]

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ABSTRACT

Given that watermelon is a crop widespread around the world, there is considerable interest in verifying how its seeds physiologically behave under unfavorable temperature conditions. This study aimed to evaluate the biochemical changes mobilization and reserves degradation, as well as the enzyme activity, during seed germination and initial growth of watermelon seedlings subjected to thermal stress, using temperature, germination percentage, germination rate and relative germination frequency over the incubation time as evaluation parameters. The experimental design was completely randomized, in a 5 x 3 factorial scheme, with five temperatures (17 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC) and three cultivars (Charleston Gray, Fairfax and Crimson Sweet), with 4 replicates of 50 seeds. Germination, seedling growth, reserve degradation and stress protection system were evaluated. Thermal stress caused deleterious effects on watermelon seeds with germination capacity at well defined temperature limits, being 25 ºC the ideal temperature, with the highest percentage of normal seedlings. Sub- (17 ºC) and supra-optimal (30 ºC) temperatures presented more than 80 % of abnormal seedlings. The α-amylase enzyme activity is intense only at the beginning of germination. Under thermal stress, the proline contents increase mainly in the cotyledons.

KEYWORDS: *Citrullus lanatus* (Thunb.) Matsum. & Nakai; enzymatic activity; germination.

RESUMO

Estresse térmico e alterações fisiológicas em sementes de melancia

Considerando-se que a melancia é uma cultura bem difundida no mundo, há grande interesse em verificar como suas sementes se comportam fisiologicamente sob condições de temperatura desfavoráveis. Objetivou-se avaliar as alterações bioquímicas de mobilização e degradação de reservas, bem como a atividade enzimática, durante a germinação de sementes e crescimento inicial de plântulas de melancia submetidas a estresse térmico, utilizando-se temperatura, porcentagem de germinação, taxa de germinação e frequência relativa de germinação ao longo do tempo de incubação como parâmetros de avaliação. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 5 x 3, com cinco temperaturas (17 ºC, 20 ºC, 25 ºC, 30 ºC e 35 ºC) e três cultivares (Charleston Gray, Fairfax e Crimson Sweet), com 4 repetições de 50 sementes. Foram avaliados a germinação, crescimento de plântulas, degradação de reservas e sistema de proteção ao estresse. O estresse térmico causou efeitos deletérios em sementes de melancia com limites de germinação em temperaturas bem definidas, sendo 25 ºC a temperatura ideal, com o maior percentual de plântulas normais. Temperaturas sub (17 ºC) e supraótimas (30 ºC) apresentaram mais de 80 % de plântulas anormais. A atividade da enzima α-amilase é intensa apenas no início da germinação. Sob estresse térmico, os teores de prolina aumentam principalmente nos cotilédones.

PALAVRAS-CHAVE: *Citrullus lanatus* (Thunb.) Matsum. & Nakai; atividade enzimática; germinação.

INTRODUCTION

The germination of non-dormant viable seeds, defined by technologists as germination and development of the essential structures of the embryo, resulting in the formation of a normal seedling, under favorable environmental conditions, is a biological phenomenon whose occurrence is determined by a set of specific environmental conditions. Among the environmental conditions that affect the germination process, temperature performs a significant influence (Marcos Filho 2015).

The gradient and thermal fluctuations to which seeds are continuously exposed constitute an important signal of the environment in controlling the different stages of the plant development. In seeds, temperature acts in both dormancy induction and breaking, as well as in embryonic growth (Bewley et al. 2013).

Temperature affects the germination process in three different ways: total germination, germination

speed and germination uniformity. Germination will only occur within certain temperature limits. Above or below this optimum temperature, there will be either reduction or non-occurrence of germination. Within these limits, there is an optimum temperature range in which the process takes place with maximum efficiency, i.e., maximum germination is obtained in the shortest time possible (Carvalho & Nakagawa 2000).

It is also crucial to consider, in addition to the minimum, maximum and optimum temperatures, the exposure duration to these conditions. Seeds present a variable behavior, in relation to temperature, and the optimum temperature could be different for various species (Custódio et al. 2002).

The germination process is complex and involves chemical reactions and many regulatory mechanisms. In most cases, the higher the temperature, the faster the germination and more efficient the process to the extent that it may affect the biochemical reactions that determine the whole germination process (Carvalho & Nakagawa 2000).

Insoluble high molecular reserves in seeds, such as lipids, proteins and sugars, are degraded and converted into soluble forms, which are then transported to growing tissues and used in the synthesis or energy production reactions (Buckeridge et al. 2004).

Metabolic modification results from a change in the whole biochemical process. Seeds, when subjected to stress, are the result of the activity of several enzymes involved in hydrolysis and transfer reactions, and can express the physiological quality of seeds, while the speed of use of the reserves may vary according to the species and environment (Bewley et al. 2013).

A high temperature stress may cause a serious perturbation to the membrane integrity (Waraich et al. 2012). The cell membranes integrity varies according to the degree of biochemical deterioration and/or physical damage, and may be considered the fundamental cause of changes in seed vigor (Vieira & Kryzanowski 1999). The process of seed germination leads to enzymatic triggers able to deploy nutritional reserves for the purpose of nutrition of the embryonic axis. Starch is amongst the reserves; and for the starch reserve to be degraded and susceptible of utilization on metabolism, it must be transformed into smaller structures, such as maltose and glucose, with the action of several hydrolytic enzymes, among them α-amylase (Buckeridge et al. 2004).

Changes and responses to thermal stress induced in the plant occur at all functional levels of the organism, which are reversible at first, but may become permanent. Even if the stress condition is temporary, the plant vitality decreases according to the stress duration (Souza et al. 2006). For this reason, this study aimed to evaluate the biochemical mobilization changes and reserves degradation, as well as the enzymatic activity, during seed germination and initial growth of watermelon seedlings submitted to thermal stress.

MATERIAL AND METHODS

The experiment was carried out at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Semiárido), in Petrolina, Pernambuco state, Brazil, in July 2011.

The experimental design was completely randomized, in a 5 x 3 factorial scheme, whose first factor corresponds to the temperatures (17 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC) and the second to the cultivars (Charleston Gray, Fairfax and Crimson Sweet).

Four replicates of 50 seeds were placed on a paper substrate (germitest) (Brasil 2009). The paper rolls were packed in plastic bags to prevent moisture loss. Subsequently, they were taken to BOD incubators regulated at temperatures of 17 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC.

The first and second germination counts were performed at 5 and 14 days after sowing - DAS (Brasil 2009), and the results expressed as average percentage of normal seedlings.

The radicle emission percentage was evaluated daily for 14 days, considering seeds with 2.0 mm of visible radicle. The germination evaluations allowed to calculate the average germination time (Labouriau 1983), average germination speed (Kotowski 1926) and germination speed index (Maguire 1962).

The average length of normal seedlings obtained from the sowing of four replicates of ten seeds was evaluated using a millimeter ruler. The average length of the seedlings was obtained by the sum of the measurements of each replicate at the 14th day, divided by the number of normal seedlings measured, with results expressed in centimeters (Nakagawa 1999). The shoot and root dry mass of four replicates of ten plants were separated into shoot and root systems and weighed in an analytical scale of 0.001 g, with values expressed in grams, before

and after drying in a greenhouse with forced air circulation at 65 ºC, until constant mass.
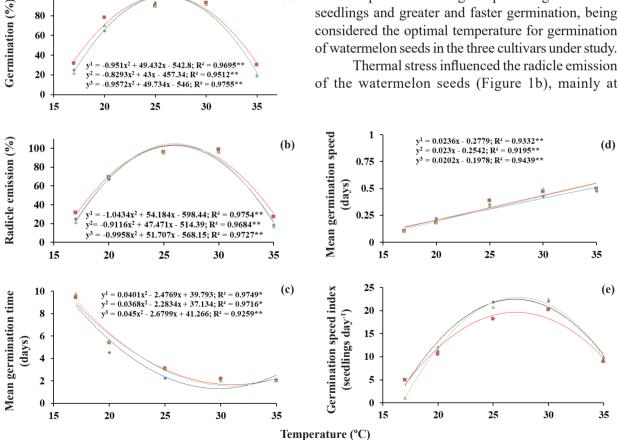
For the biochemical assays, seed cotyledons were collected from seedlings at 1, 3 and 5 DAS (Brasil 2009) and maintained at temperatures of 17 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC, with cell contents extracted in potassium phosphate buffer (100 mmol L^{-1}) at pH 7.0. The homogenate was centrifuged at 10.000 x g for 20 min, at 4 ºC. The supernatant was removed and placed in microtubes and kept in a freezer at -20 ºC. Total soluble sugars (Morris 1948, Yemm & Willis 1954), reducing sugars (Miller 1959) and total soluble proteins (Bradford 1976) were quantified.

The extraction and quantification of α-amylase activity (EC 3.2.1.1) were performed according to Aragão et al. (2003) and the determination of proline content was conducted based on the methodology described by Bates (1973).

The data obtained for germination and initial growth were submitted to variance analysis and the means compared by regression analysis at a 0.05 significance.

RESULTS AND DISCUSSION

The results showed significant effects of the interaction between cultivar and temperature for all variables, germination, germination kinetics, reserves degradation and mobilization, α-amylase activity and proline amino acid content. Regardless of the variety evaluated, there was a difference among the temperatures (Figure 1a); and the percentage of normal seedlings gradually increased from 17 ºC to 25 ºC. Thus, at the lowest temperature (17 ºC), germination was between 21 % and 31 %, while, at the highest temperature (35 ºC), germination was delayed, but not interrupted. Sub- (17 ºC) and super-optimal (35 ºC) temperatures presented 100 % and over 80 % of abnormal seedlings, respectively. The temperature of 25 ºC provided the highest percentage of normal seedlings and greater and faster germination, being considered the optimal temperature for germination of watermelon seeds in the three cultivars under study.

Thermal stress influenced the radicle emission of the watermelon seeds (Figure 1b), mainly at

Figure 1. Germination, radicle emission, mean germination time, mean germination speed and germination speed index of three watermelon cultivars [Charleston Gray (green); Fairfax (red); Crimson Sweet (blue)] submitted to different temperatures. ** Significant at 1 %.

temperatures of 17 ºC and 20 ºC, where germination was delayed because of the low temperatures and, consequently, slow imbibition. In turn, at 25 ºC and 30 ºC, a higher radicle emission percentage was observed.

As the temperature increased, there was a reduction in the average germination time (Figure 1c), and thus, at the minimum temperature (17 ºC), the watermelon seeds showed an average germination time of approximately nine days. In turn, under the maximum temperatures of 30 ºC and 35 ºC, they had an average time of two days.

The average germination speed increased according to the increase in temperature (Figure 1d). Thus, slower germination rates are in the range between 17 ºC and 20 ºC, whereas the range between 30 ºC and 35 ºC is considered optimal for these cultivars.

The germination index showed a similar behavior to the average germination speed, increasing as temperature increased (Figure 1e).

As for shoot and root length, the shoot and root dry mass could not be evaluated at the maximum temperature of 35 ºC, because, at 14 DAS, the seedlings were dead, rotten and with an intense attack of fungi.

In relation to shoot length, the values were similar among the cultivars, varying from approximately 3.0 cm to 9.0 cm, at temperatures of 17 ºC to 30 ºC, respectively (Figure 2a).

All cultivars presented the same behavior for root length (Figure 2b), increasing the average values as temperature increased. When the watermelon seeds were subjected to a temperature of 30 ºC, these values were higher than the others, presenting the highest root lengths for the cultivars Fairfax, Charleston Gray and Crimson Sweet, respectively.

The watermelon cultivars Charleston Gray and Fairfax presented a similar behavior, regarding the shoot dry mass (Figure 2c), showing a greater difference for the temperature of 30 ºC, in which the results were inferior to 20 ºC and 25 ºC. Lower shoot dry mass values were observed for the Crimson Sweet cultivar at all temperatures.

Regarding the root dry mass, the cultivars presented a similar performance (Figure 2d), and the highest and lowest temperatures provided the lowest amounts of root dry mass, showing that thermal stress, besides affecting the germination process, also affects the growth and development of watermelon seedlings. For the three cultivars, the temperature of 25 ºC yielded the largest amount of root dry mass.

As it can be observed, the average sugar content of the cotyledons did not differ significantly
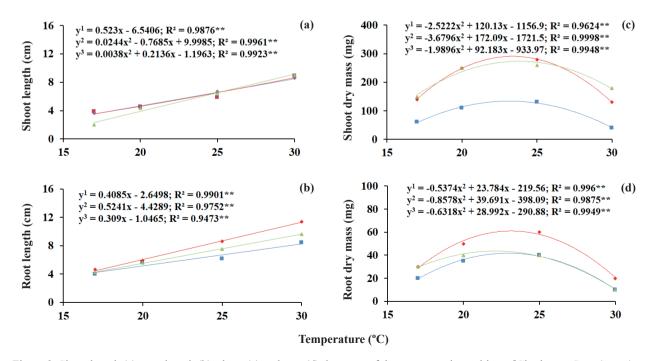

Figure 2. Shoot length (a), root length (b), shoot (c) and root (d) dry mass of three watermelon cultivars [Charleston Gray (green); Fairfax (red); Crimson Sweet (blue)] subjected to different temperatures. ** Significant at 1 %.

between cultivar and temperature, during the imbibition period (Figure 3), but from radicle protrusion to the seedling stage.

The total soluble sugar contents decreased in the cotyledons for all varieties during the initial germination stage, increasing later (Figure 4). It is possible to observe that there is a greater accumulation of total soluble sugars at the temperature of 25 ºC and that the stresses caused by the smallest and highest temperatures present smaller amounts of carbohydrates, respectively.

The α-amylase enzyme activity increased at the beginning of the germination process up to 3 DAS, at all temperatures, decreasing at 5 DAS, as the germination process progressed (Figure 5).

The average protein content in the cotyledons changed during the germination process for the three cultivars. An increase was observed during the imbibition and radicle emission, with a consequent reduction in the period of seedling formation (Figure 6). As temperature increased, there was

also an increase in the total protein content, since at the temperature of 30 ºC the highest values were observed, and at 20 ºC the lowest values were observed.

With the increase in temperature, there was an expressive increase, especially up to 25 ºC, in the proline contents of the cotyledons collected at 5 DAS, showing the same trend for the cultivars Charleston Gray (Figure 7a), Fairfax (Figure 7b) and Crimson Sweet (Figure 7c). The proline concentration also increased in embryonic axes with the increase in temperature, but its content was lower than that of the cotyledon for all cultivars.

The optimum temperature provides the maximum germination percentage and speed (Oliveira et al. 2014, Pinheiro et al. 2014). Low temperatures delay the metabolic rate to a point where reactions that are essential to the onset of germination would no longer occur, drastically compromising this process and in less time. On the other hand, high temperatures cause a thermal stress to the seeds,
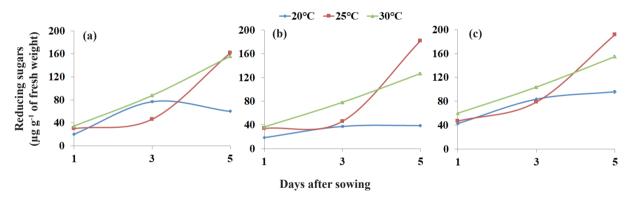

Figure 3. Amount of reducing sugars in the cotyledon at one, three and five days after sowing of watermelon seeds (*Citrullus lanatus*) of the Charleston Gray (a), Fairfax (b) and Crimson Sweet (c) cultivars subjected to thermal stress.
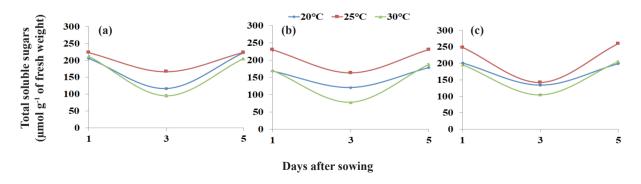

Figure 4. Amount of total soluble sugars in the cotyledon at one, three and five days after sowing of watermelon seeds (*Citrullus lanatus*) of the Charleston Gray (a), Fairfax (b) and Crimson Sweet (c) cultivars subjected to thermal stress.

leading to thermal inhibition, thermal dormancy or even loss of viability (Bewley et al. 2013). In the present experiment, the seeds showed a maximum germination percentage and speed at intermediate temperatures, with low values at extreme (high and low) temperatures. This large temperature range may contribute to a better adaptation of these cultivars to variable environments.
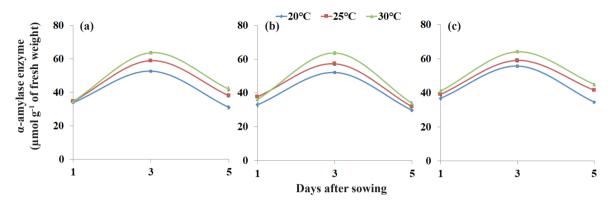

Figure 5. Activity of the α-amylase enzyme on the cotyledon at one, three and five days after sowing of watermelon seeds (*Citrullus lanatus*) of the Charleston Gray (a), Fairfax (b) and Crimson Sweet (c) cultivars subjected to thermal stress.
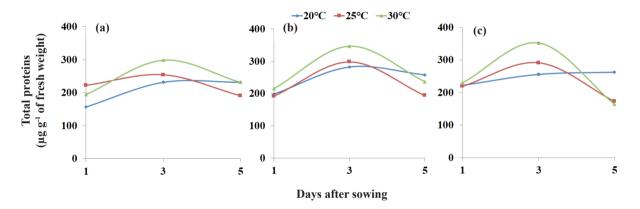

Figure 6. Amount of total proteins in the cotyledon at one, three and five days after sowing of watermelon seeds (*Citrullus lanatus*) of the Charleston Gray (a), Fairfax (b) and Crimson Sweet (c) cultivars subjected to thermal stress.
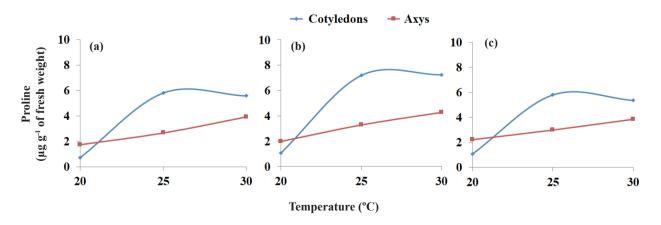

Figure 7. Amount of proline in the cotyledon (blue) and axis (red), at five days after sowing watermelon seeds (*Citrullus lanatus*) of the Charleston Gray (a), Fairfax (b) and Crimson Sweet (c) cultivars subjected to thermal stress.

Regarding root emission, at 30 ºC, a primary root started to be emitted, but its subsequent development was limited, followed by deterioration of the seeds, release of exudates in the germinative medium and fungal growth on the roots surface. This fact may be explained by the high temperatures, which contribute to the deterioration of the seeds, as observed by Silva Bello et al. (2008) in cherry seeds, at 40 ºC. Radicle emission was not negatively affected at 25 ºC.

As for the average germination time, when seeds are imbibed at sub-optimal temperatures, such as 15 ºC and 20 ºC, injuries may occur due to thermal stress, probably related to the damages caused in the membrane system, which disturb the seed germination and seedling establishment (Filgueira 2000). Within the 25 ºC temperature range, germination speed is more orderly; and above or below this range, the values increase or decrease significantly.

At 17 ºC, a minimum speed index was observed, and, at 30 ºC and 35 ºC, a maximum speed index was evident; however, the temperature of 25 ºC showed an intermediate speed index. Although the germination was stimulated at temperatures higher than 25 ºC, the germination process was subsequently inhibited, abruptly preventing the formation of normal seedlings and favoring seed deterioration by the presence of fungi. At these elevated temperatures, the formation of abnormal seedlings, characterized by a great occurrence of weak and/or atrophied roots, was observed. The temperature of 25 ºC is the most adequate for this variable.

At lower temperatures (17 ºC and 20 ºC), the root growth of the cultivars was affected, showing the lowest plant heights (average of 3.0 cm), differing from the temperature of 30 ºC, at which all cultivars had longer shoots (9.0 cm). This reduction in growth at low temperatures may be attributed to the inhibition of the enzyme activity in the seedling metabolism (Sassenrath & Ort 1990), respiratory rate reduction (Berry & Raison 1981), decrease in the photo-assimilate translocation in the phloem (Giaquinta & Geiger 1973) and direct and differential effect of low temperature on cell expansion, which would be reduced by the effect of cold on the cell wall, thus increasing its stiffness (Pollock 1990).

The highest root lengths, when watermelon seeds were subjected to a temperature of 30 ºC, were evidenced in the present study (Figure 2b), being more influenced by the temperature elevation than the total germination percentage (Figure 1a).

The decrease in shoot dry mass at elevated temperatures may be related to the fact that, with the increase in temperature, there is also an increase in breathing, given that watermelon is a C_3 plant, i.e., typically sensitive to high temperatures (Taiz & Zeiger 2004).

At 20 ºC, the reducing sugar contents increase from imbibition until primary root emission and decrease when forming the seedling, and, at 30 ºC, there is only an increase trend. In turn, at 25 ºC, these contents are practically constant until the radicle emission stage, increasing significantly during seedling formation to the days after sowing, with a tendency of mobilization of these reserves during this period. This accumulation of sugars is a common feature in the cells of organisms under increases of temperature or actually stressed, since sugars have a role in osmotic adjustment, and also have indirect protective effects, such as protein stabilization (Bianchi et al. 1991).

There was a decrease and a subsequent increase in the total soluble sugar contents in the cotyledons (Figure 4). It is also possible to observe that there is a greater accumulation of total soluble sugars at the temperature of 25 ºC. Apparently, the mobilization of these reserves occurs after the radicle protrusion, as shown by Sassaki & Felippe (1992). As the reserves in storage organs are mobilized, they are converted into forms that are readily transportable to sites of need (usually growing organs). Reserve mobilization decreases as the seedling emerges above the soil and becomes photosynthetically active (Bewley et al. 2013).

As regards the α-amylase enzyme activity with the increase at the beginning of the germination process, the highest enzyme activity occurred at three days and the lowest at five days after germination, possibly due to the depletion of the starch reserves in the endosperm of the seeds. It is thus verified that α-amylase has a high activity at the beginning of the germination process, and, over time, it is reduced. This behavior is due to the reduction in the starch content as germination progresses (Bewley et al. 2013).

The decrease in the protein concentration is probably due to the mobilization of nitrogen reserves, since proteins are composed of reserves in seeds that have nitrogen in their composition. The total protein content in the seeds increased during the imbibition process, and such an increase was associated with the induction of biosynthesis of hydrolytic enzymes

(Pontes et al. 2002) and response to temperature, given that high temperatures inactivate enzymes, change lipid conformation and de-structure proteins; as well as peptide conformation changes the de-structuring of protein complexes in the cell membrane (Jansen & Ison 1994).

In relation to the increase in amino acid proline in this study, plants alter the metabolism in various ways to accommodate environmental stresses, including thermal stress, producing osmoregulatory compounds such as the amino acid proline (Taiz & Zeiger 2004). In general, high temperatures lead to a decrease in the amino acid supply, RNA and protein synthesis, as well as a decrease in the rate of metabolic reactions (Riley 1981), which may retard or suppress germination and even cause loss of viability.

CONCLUSIONS

1. Watermelon seeds present a germination capacity at well defined temperature limits, being 25 ºC the ideal temperature;
2. The α-amylase enzyme activity is intense only at the beginning of germination;
3. Stress factors owing to increased proline contents occur specifically in the cotyledons.

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