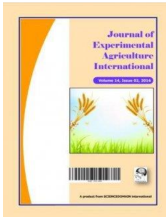

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Physiological Changes in Seeds of *Solanum paniculatum* L. during Heat Stress

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Physiological changes occur in seeds before and during the germination process when subjected to high temperatures. Considering that *Solanum paniculatum* presents an adaptation to different environments, the aim of this study was to evaluate physiological changes of level of heat stress tolerance in seeds of *S. paniculatum*, before and during germination. Four-time periods (12, 24, 48 and 72 h for the 45°C temperature) were tested before and after the germination process in seeds of *S. paniculatum*. The degree of humidity, imbibition curve, percentage of germination, mean germination time (MGT) and germination uniformity coefficient (T7525), relative frequency at different times of temperature exposure of 45°C. The seeds of *S. paniculatum*, characterized in the period of 432 h (18 days), present the three-phase water absorption pattern. Seeds submitted to heat stress during the germination process presented higher tolerance than those submitted to high temperatures before imbibition, suggesting greater stability of the physiological constituents of the seeds to stress. Germinability of *S. paniculatum* can be intensified even under stress and suggests a broader frequency distribution than seeds that did not undergo stress during germination.

Keywords: Seeds vigor; oxidative damage; stress tolerance; germination process.

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1. INTRODUCTION

Due to the diversity of Brazilian climatic conditions; temperature variations in some regions can cause stress in plants and alter physiological and biochemical processes before and during seed germination [1]; There are several causes that affect the physiological quality of seeds while remaining in the soil over time; which are exposed to a series of environmental factors that provoke stress reactions; biotic (fungal and insect attack) or abiotic (exposure at high temperatures and water restriction); whose changes that can reflected in the form of stress; and they can cause damages to the seeds [2-4].

The temperature has significant influence among the environmental conditions that affect the germination process [5]. Thus, high temperatures affect seeds in embryonic growth, induction and dormancy breakdown [6], and studies are required to simulate adverse environmental conditions in the understanding and identification of the responses to this type of stress in plants.

The seeds subjected to stress conditions are able to modify the metabolism, changing the whole biochemical system, as a result of the activity of several enzymes involved in the hydrolysis and transfer reactions, which can express the physiological quality of the seeds, and the rate of utilization of seed reserves. It may vary according to the species and the environment to which they are inserted [6,7].

Changes and responses to plant-induced thermal stress occur at all functional levels of the organism, which are reversible at the onset but may become permanent [7]. One of the techniques that can be used in studies about the identification of levels of tolerance to thermal stress is the exposure of seeds at different times in high temperature, however, little is known about the physiological responses during the stress caused in the germination process [8]. A few types of research were done with solanaceous species; one of that didn’t succeed to find physiological parameters studying germination process to improve the seedlings quality in the field [9]. Thus, it is important to select species according to the type of mechanism developed on high-temperature conditions.

Solanum paniculatum is a species of solanaceous family native from Brazil, which has great importance to the food and medical industry, adapting to the more inhospitable environments, such as the occupation of high-temperature sites [10,11], and therefore it is indicated to compose poor arable areas, serving as a model species in this study.

Thus, the aim of this study was to evaluate the tolerance level in *S. paniculatum* seeds to heat stress, before and during the germination process.

2. MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Analysis of Forest Seeds in the Forest Engineering Department of the State University of São Paulo (UNESP), Campus Botucatu-SP. The seeds of *Solanum paniculatum* were collected in August 2012 in the region of Lavras - MG, they were benefited and stored under controlled conditions in cold room at 5 °C and 60% of relative humidity (RU).

The moisture content of the seeds was determined by adopting the oven method at 105±3°C for 24 hours, as recommended by the Rules for Seed Analysis [12]. The calculation was done on a wet basis, and the degree of humidity is expressed as a percentage.

The percentage of water acquisition was calculated in relation to the initial weight of the seeds of each treatment. Before starting water acquisition, the seeds were weighed on a digital scale with accuracy of 0.0001 g and then four replicates of 0.5 g of *S. paniculatum* seeds were placed to soak in Petri dishes, placed on two filter paper sheets, moistened with distilled water, i.e. 2.5 times the weight of the dry paper sheets [11] and maintained at temperature of 20-30°C in a BOD-type germination chamber. The seeds were weighed at two-hour intervals during the first 12 hours and then at 12 hours intervals until they reached 50% germination and/or until the 20th day after water acquisition begin. It is important to note that, before each weighing, the seeds were surface dried with absorbent paper and then replaced under the conditions of germination.

For the germination test, the same conditions described in the previous experiment were performed. Before germinating the seeds, the seeds were immersed in 1% hypochlorite solution for five minutes for disinfestation, and then placed on two sheets of paper moistened with water in Petri dishes and kept in BOD type germinator at 20-30°C for 21 days.

To study the germination of *S. paniculatum* seeds under different temperature expositions, constant time periods: 12, 24, 48, 72, 120 and 240 hours at 45°C temperature were tested before and after the germination process. The treatments were represented by the different time exposure period. Thus, pre-germination treatments in dry seeds (before the germination process), to analyze the time of tolerance to thermal stress, consisted of seeds without stress treatment and 12, 48, 72, 120 and 240 hours incubated at 45°C.

For germination tests at tolerance to thermal stress, soon after being submitted to the stress treatments, the seeds were sowed in Petri dishes on filter paper, moistened with water in an amount corresponding to 2.5 times the mass of the dry substrate, and they were placed in a germination chamber type BOD, with temperature set at 20-30°C, with a 12 hour of photoperiod, ideal temperature for germination of the species [12].

The experimental design was completely randomized, with four replicates of 25 seeds for each treatment. Daily evaluations were carried out, with germination criteria being the radicle emission. Germinator software [13] and SISVAR [14] were used for the calculation of mean germination time (MGT) and germination uniformity coefficient (T7525).

3. RESULTS AND DISCUSSION

The seed imbibition curve of *S. paniculatum*, was characterized in the period of 432 hours (18 days). The curve showed changes in the three physiological phases, allowing the graphic visualization of the three-phase pattern of water acquisition by the seeds, which can be denoted three differentiated stages throughout the germinative process (Fig. 1).

It is possible to verify the evolution of the germination process of *Solanum paniculatum seeds*, which assumes the three-phase pattern of the imbibition curve initiated in phase I, which is the time zero (0) and extends to the first inflection point of the characterized curve by intense inflow of water due to the difference in matrix potential of the seed tissues, which started after 2 h at the beginning of the imbibition, with a mass increase of 33% of the initial weight, and this mass stabilized at the initial 4 h at the beginning of the imbibition, whose percentage of mass gain was equivalent to 43%, that is, an average increase of 10.7% per hour of imbibition. Phase II, which is the transient period between the rapid water uptake between the two inflection points of the curve, is characterized by the lower rate of water absorption, but with increasing seed weight; in this phase the water absorption was less intense between 6 and 240 hours of absorption, which could be characterized by the beginning and the end of phase II of the germination. Phase III is characterized by a new increase in water absorption by the seed just after 240 h, a period corresponding to a more expressive gain of mass also characterized by the protrusion of the radicle.

The initial rapid imbibition of the seeds acquiring the three-phase imbibition pattern indicates that

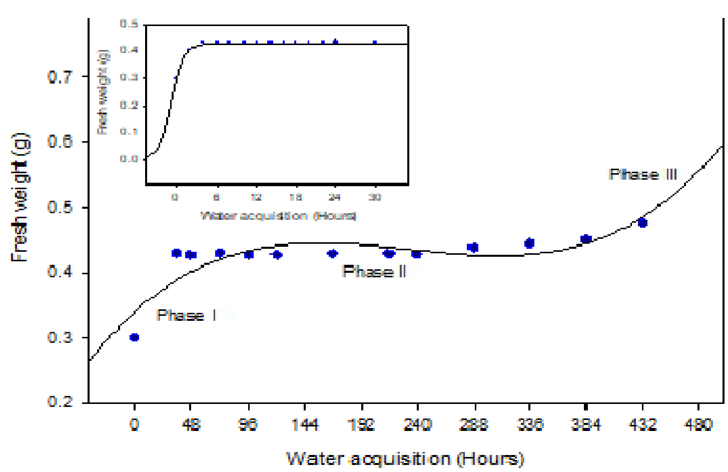

Fig. 1. Water acquisition curve of *Solanum paniculatum* referring to the weight of the fresh matter (g) along 432 hours; in the inset refers to the water acquisition along 30 hours

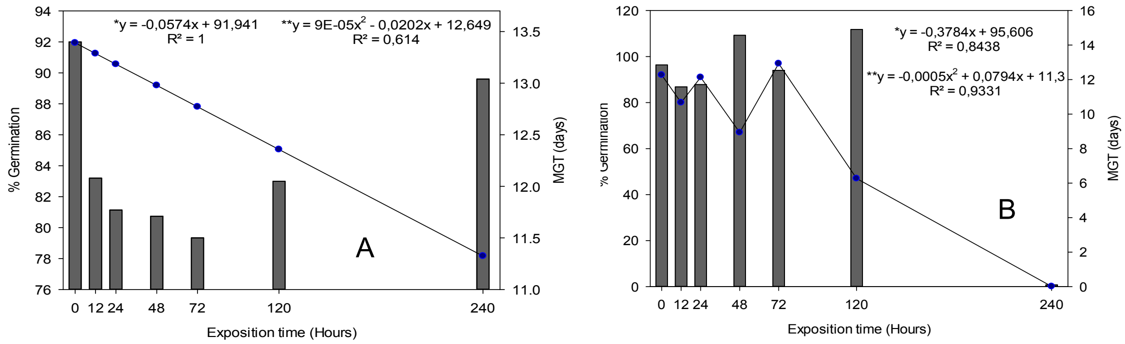

Fig. 2. Effect of germination and mean germination time (MGT) on seeds of *Solanum paniculatum* when exposed to thermal stress at 45°C (A) before germination and (B) during germination. * eq. Germination; ** eq. MGT

this species has no physical dormancy [6,15] since at 18 days after the beginning of imbibition the average percentage of germination was of 87% of the seeds.

The germination of the *S. paniculatum* species after exposure to the 45°C temperature decreased linearly, but the values remained around 78% germination even after 240 hours of thermal stress (Fig. 2A). On the other hand, when stressed during the germination process, the effects were variable, so that, after 120 and 240 hours of exposure, germination was 45% and 0%, respectively (Fig. 2B).

The seeds that underwent thermal stress before being placed to germinate were able to maintain high germination (Fig. 2A). This can be explained by the high tolerance of the tissues given to the seeds of this species since they did not suffer physiological damages to the internal structure of the seed because they are not yet hydrated. However, according to the work of Alves et al. [16] studying the effect of high temperatures on seeds of *Bauhinia divaricata* immersed in hot water at 80°C, was reported that there was a low percentage of germination due to the internal structure of the seeds presenting some kind of physiological damage. This caused the death of the seed embryo due to high temperature, which did not happen in the present study in *S. paniculatum* seeds.

The seeds of *S. paniculatum* when subjected to a heat stress temperature (45°C) during the germination process, were observed low germinative percentage, followed by null values of germination (Fig. 2B). The non-emergence of seedlings when the seeds are exposed to high temperatures can be explained by the damage of essential seed structures; germination and/or radicle protrusion may have started, but it is not enough for the seedlings to emerge [17].

The high percentage of germination obtained up to 240 h (Fig. 2A) and 120 h (Fig. 2B), due to the type of stress submitted, can be verified in other species, as reported by [17], in studies with seeds of *Bauhinia divaricata*, verified the percentage of optimum germination at 25°C, decreasing consecutively. Likewise, [17] studied the effect of temperature on seeds of germinated *Bauhinia forficata* observed that until the temperature of 40°C this species can germinate and from that temperature, there is a decrease in germination.

It was observed that the MGT of seeds exposed to stress before the germination process was the lower when exposed to a temperature of 72 h (Fig. 2A); however, seeds exposed to stress during the germination process had the lowest MGT at a temperature of 12 h (Fig. 2B). This may have been due to damage to the seeds at high temperatures, reducing the percentage of germination.

When the seeds were exposed to high temperature, the uniformity remained stable up to 48 hours, shortly after this period the uniformity decreased until the last analyzed period (240 h) (Fig. 3A). On the other hand, when the seeds were exposed to high temperature during the germination process, the uniformity extended up to 120 hours, and soon thereafter, a decrease in uniformity was observed (Fig. 3B).

The seeds of *S. paniculatum* when exposed to thermal stress at 45°C during the germination process showed a greater and more variable germinative uniformity (Fig. 3). According to Carvalho et al. [18], Borghetti [19], the variation in the uniformity of germination of seeds in detriment to high temperatures by prolonged exposure time can be explained due to the denaturation of proteins, with consequent changes in the enzymatic activities that are necessary for germination, causing severe damages in the cellular structures, often resulting in delay and/or prevention of germination.

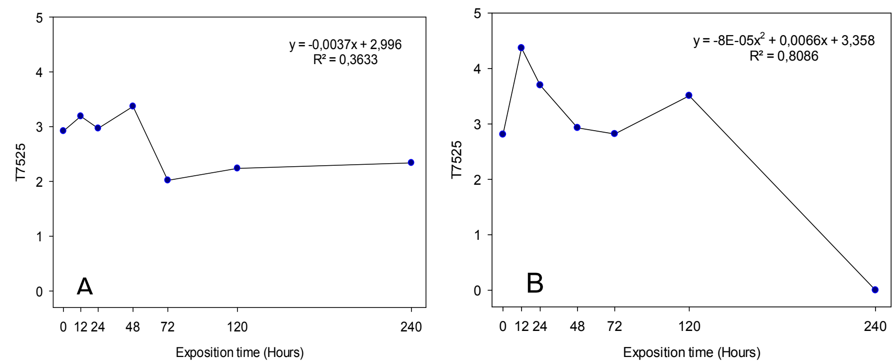

Fig. 3. Effect of thermal stress on the uniformity of germination in *Solanum paniculatum* seeds when exposed to thermal stress at 45 °C (A) before the germination process and (B) during the germination process. * eq. Germination; ** eq. MGT

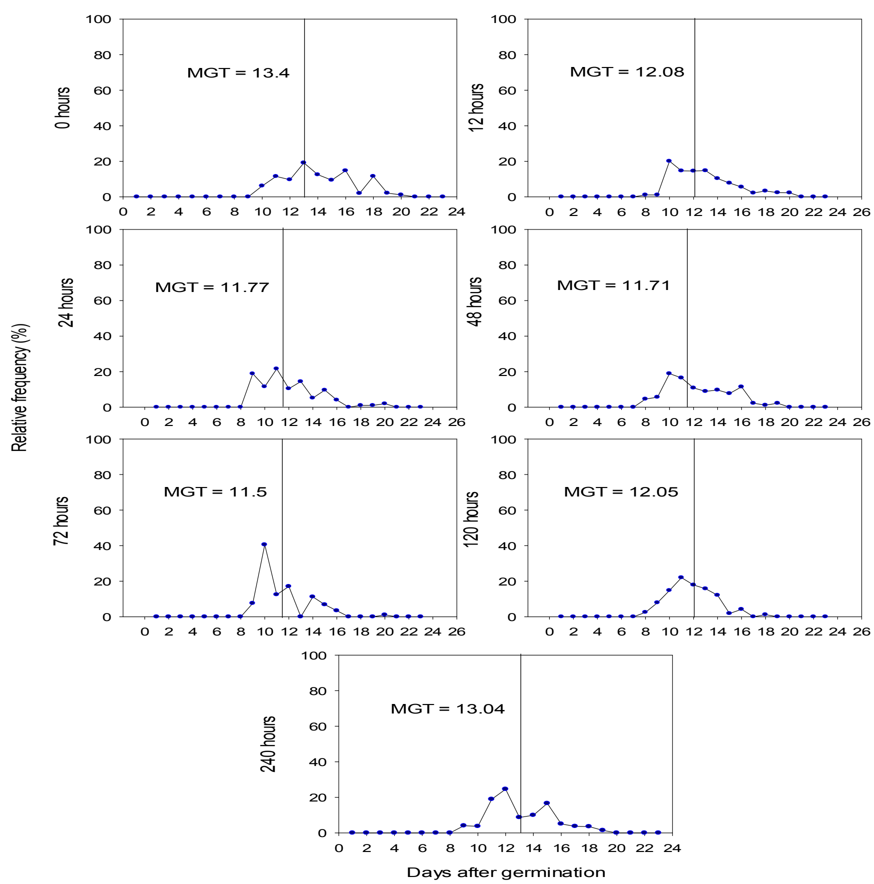

Fig. 4. Relative frequency (%) of seeds of *Solanum paniculatum* under conditions of thermal stress before the germination process

After dispersion, seed germination is normally non-uniform in order to increase the survival success of the species in the field [20-21]. Thus, according to the results of this research, it is observed that the heat stress after the beginning of the germination process in *S. paniculatum* increases the variability of the uniformity, which can modify the behavior of the species to support abiotic stresses that threaten its survival [22].

The relative frequencies of the average germination time of *S. paniculatum* seeds, under thermal stress conditions before and during the germination process, show variation according to the time of exposure of the seeds (Figs. 4 and 5).

For the *S. paniculatum* species, mean germination time (MGT) decreased from the exposure to stress, reaching the lowest value in 72 h of exposure, as 11.5 days, which was accompanied by a decrease in germination in this period, however, between 120 and 240 h there was MGT increase, reaching 13 days (Fig. 4). On the other hand, when analyzed the effect of stress during the germination process, it is observed that the MGT remains practically unchanged, between 0 and 120 hours of exposure, with values varying between 12 and 15 days (Fig. 5).

It is also verified that the stress levels in the pattern of distribution of the polygons of relative frequency in the germination of *S. paniculatum* seeds present a unimodal pattern both for seeds that underwent stress during the germinative process and those that did not suffer. As the stress levels increase, the distribution of germination remains stable. In general, the maximum frequency distribution frequency was 20%, except for seeds with 72 hours of exposure to stress, which presented 40% (Figs. 4 and 5).

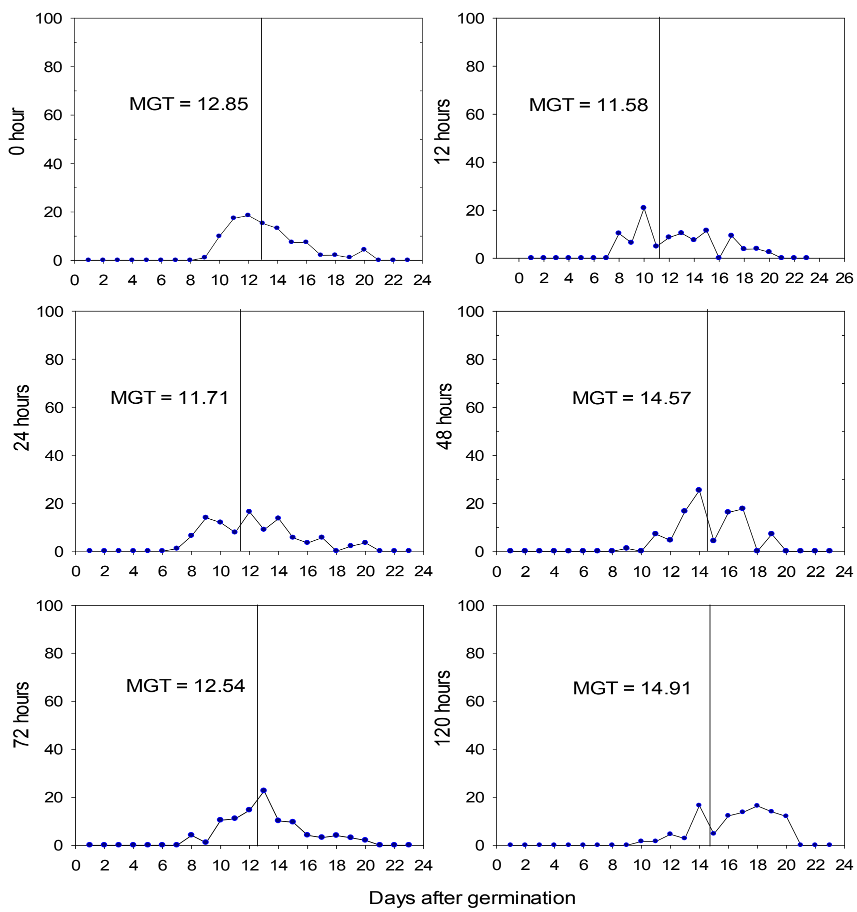

Fig. 5. Relative frequency (%) of seeds of *Solanum paniculatum* under conditions of thermal stress during the germination process

With the application of seed stress levels during the germination process, the frequency polygons moved to the left, indicating that the germination can be intensified even under stress. However, the seeds that underwent stress during the germination process, in general, the frequency polygon takes off slightly to the right, which suggests a broader frequency distribution than that observed in seeds that did not undergo stress during germination (Figs. 4 and 5).

It is important to emphasize that temperatures below or above optimum are able to reduce the germination speed, as well as MGT, by exposing germinated seedlings to adverse abiotic factors for a longer time, consequently reducing germination. High temperatures are able to reduce the viscosity and increase the kinetic energy of the water inside the cells, allowing a fast absorption by the seeds and greater speed in the reactions; the reduction of the temperature causes a significant decrease in the germination speed, due to the effects generated on the mobilization of reserves and the time of imbibition [23,15,5].

4. CONCLUSIONS

The heat stress during germination in *S. paniculatum*, despite decreasing the viability of the seeds, it is accompanied by the stability of some physiological characteristics, such as MGT, Fr (%) and T7525, which suggests elevated tolerance to high temperatures even after the onset of germination process.

During the germination process, exposure seeds to elevated temperature decrease the viability of the seeds, suggesting the germination process and defense response to heat stress are independent physiological aspects of *S. paniculatum* behavior in the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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