Thermal-biological aspects on the seed germination of *Cucumis anguria* L.: influence of the seed coat

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ABSTRACT - (Thermal-biological aspects on the seed germination of *Cucumis anguria* L.: influence of the seed coat). The seed coat influences the early stages of germination of many seeds and sometimes maintains seed dormancy. Early reports have shown that the testa influences the germination response of *Cucumis anguria* seeds to light although the response to temperature as influenced by the tegument is not well understood. The main purpose of this study was to observe the influence of the testa on the germination of *Cucumis anguria* by using parameters as germinability and isothermal germination rate. The assays were carried out in a thermal-gradient block with water imbibed seeds kept in darkness. Estimates of the activation enthalpies (ΔH) show $|\Delta H| < 50$ kJ.mol^{-1} between 26.1 °C and 35.2 °C (intact seeds) and between 25.4 °C and 35.2 °C (scarified seeds), whereas at temperatures greater than 35.2 °C the germination may be limited by processes with $|\Delta H| > 125$ kJ.mol^{-1}. It is suggested that the testa limits embryo expansion rather than interfering with diffusion processes.

RESUMO - (Aspectos térmico-biológicos da germinação de sementes de *Cucumis anguria* L.: influência do tegumento). O tegumento pode influenciar a germinação e a dormência de sementes. Trabalhos anteriores mostram que a testa influencia a resposta germinativa de sementes de *Cucumis anguria* à luz, embora a influência do tegumento na resposta à temperatura ainda não esteja bem estabelecida. O principal objetivo deste estudo foi observar a influência da testa na germinação de *C. anguria*, usando-se como parâmetros a germinabilidade e a taxa de germinação isotérmica. Os ensaios foram realizados em bloco de gradiente térmico, em condições de escuro constante. Estimativas da entalpia de ativação (ΔH) mostram $|\Delta H| < 50$ kJ.mol^{-1} entre 26,1 °C e 35,2 °C (sementes intatas) e entre 25,4 °C e 35,2 °C (sementes escarificadas), e sugerem a ocorrência de processos com $|\Delta H| > 125$ kJ.mol^{-1} em temperaturas acima de 35,2 °C. Os resultados sugerem que a testa pode limitar a expansão de embrião, não interferindo em processos de difusão.

Introduction

Seed germination starts with imbibition and finishes with primary root, cotyledon or coleoptile emergence from the seed coat. The seed coat may influence the early stages of germination by establishing a permeability barrier and interfering with diffusion processes such as water uptake and gaseous exchange, or by exerting a mechanical constraint on expansion by the embryo (Mayer & Shain 1974, Welbaum *et al*. 1998).

Temperature affects the germination capacity (germinability), the germination rate, and the distribution of the relative frequency of germination along the incubation time (Labouriau 1978, Kocabas *et al*. 1999). The thermal limits for germination are defined by an optimum T_o, a minimum T_m, and a maximum T_M, temperature which can characterize some of the ecological limitations for the geographic distribution of the species. The rate parameters allow the evaluation of some physiological mechanisms controlling seed germination through the treatment based on the absolute reaction rate theory. Accordingly, the isothermal germination should be considered as involving the activation of a substratum and its transition from a ground state to a final state of the process (Hageseth & Young 1994). If the proportionality between the rate of the reaction and the constant of chemical balance (k) among the initial and final states of the system is considered and if the effect of temperature on k is determined by ΔH (the net enthalpy variation) of the reaction, ΔH determines the effect of temperature on the rate of the germination process. It may be possible to analyse ΔH values since they are coupled to changes in the thermodynamic equilibrium between the ground state and the activated state in a protein reaction (Oliveberg *et al*. 1995).

It has been reported in watermelon seeds that higher germination at low temperatures can be obtained by seed coat splitting (Nerson *et al*. 1985).

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According to Edelstein & Kigel (1990), certain cucurbit seeds, such as *Cucumis melo*, require relatively high temperature for successful germination, but several types of seed treatments can improve low-temperature germination. Those authors reported that seed coat plays an important role in the germination response to low temperatures in *Cucumis melo* seeds, with decoating being more effective than splitting the seed coat at the radicular end of the seed in relieving inhibition of germination.

Cucumis anguria L., known as indian gherkin, originated in Africa and is widely distributed in the north, northeast and southeast of Brazil. Cardoso & Felippe (1988) reported that germination of *Cucumis anguria* is light-inhibited and the seed coat may act as light filter favouring the transmission of longer wavelengths. Seed scarification on the micropyle region completely overcame the inhibitory effect of white light and the maximum germination values were similar to those corresponding to the dark control (Cardoso & Felippe 1988). The effect of temperature on tegument properties controlling germination of *Cucumis anguria* seeds is not well understood. In the present study the interaction between testa and temperature on the germination of that species was observed taking into account the temperature dependence of the germinability and of the germination rate, to know whether the action mechanisms through which the testa influences the germination of *Cucumis anguria* and to provide addition information on factors limiting germination of a tropical cucurbit seed.

Material and methods

Plant material - Seeds of *Cucumis anguria* L. (Cucurbitaceae) used in the experiments were harvested at Instituto de Biociências, UNESP, Rio Claro, SP, Brazil, in 1996/97. Fruits were collected once ripe and seeds were separated from fruits with aid of a sieve. The seeds were washed in tap water to remove the remaining pulp, dried to nearly 8% moisture content under the sunlight at room temperature and stored in glass jars kept in darkness at temperatures ranging from 25 °C to 30 °C.

Germination assays - The germination assays were performed in a temperature gradient block based on methodology described by Labouriau & Agudo (1987). All the assays were repeated twice. The temperatures were measured with PT100 sensors (JK Instrumentos, ME, Piracicaba, SP) connected to an electronic thermometer JK model SK 010 (JK Instrumentos, ME, Piracicaba, SP). Seeds were germinated in plastic trays lined with strips of Whatman N° 1 filter paper kept saturated with distilled water; the trays were placed inside

glass assay tubes (250 x 25 mm) closed with stainless steel stoppers. Five tubes per treatment (40 seeds per tube) were used, and the assays were kept in darkness throughout.

Seeds were considered germinated once the radicle emerged and had geotropic curvature. Observations were made at 24 h intervals under dim green light, and germinated seeds were withdrawn as soon as recorded.

Scarification involved the removal of a piece (about 1 mm^2) of the testa near the micropyle of unimbibed seeds with aid of a razor blade.

Analyse of data - The final percentage of germinated seeds (G) was transformed in angular values and the optimum germinability was found by searching for a temperature interval in which the regression line of the transformed germinabilities was parallel to the temperature axis. Confidence intervals ($\alpha = 0.05$) were determined for G to search for the upper and lower temperature limits, in which the confidence intervals intercept the temperature axis (Labouriau & Osborn 1984).

The rate parameters were computed as follow (Labouriau & Osborn 1984): a) average isothermal germination rate, $v = 1/t$, with t, days, $= \Sigma(n_i.t_i)/\Sigma(n_i)$, where n_i is the number of germinated seeds between two consecutive observations t_{i-1} and t_i; b) the time variance, $s^2_t = \Sigma n_i(t_i-t)^2/ (-1 + \Sigma n_i)$; c) the rate variance, $s^2_v = (v)^4.s^2_t$ and; d) the weighted average rate, $V = \Sigma w_j.v_j/\Sigma w_j$, with $w_j = n_j /(s^2 v_j)$, n_j = number of germinations in the jth replication, and $s^2 v_j$ = variance of the germination rate of jth replication. Germination rates were compared by stepwise exhaustive comparisons of pairs of isotherms (two-tailed Wilcoxon test) (Sokal & Rohlf 1995).

The distributions of isothermal germination frequencies (f_i) were computed from the formulae $f_i = n_i/\Sigma n_i$. The uncertainty, U, was computed as $U = -\Sigma f_i.log_2 f_i$, in bits, and represents Shannon's informational entropy, which measures the degree of synchronisation of germination (Labouriau & Agudo 1987).

The enthalpy change of activation (ΔH) of the germination was estimated from the slopes $[\partial(-R.lnV)/\partial(1/T)] = \Delta H + RT$ of the Arrhenius graph, $-R.lnV$ versus $1/T$, with $R = 8.314$ J(Kmol)$^{-1}$, V = weighted average rate and T = temperature in K (Labouriau & Osborn 1984).

Results

Germination of intact seeds incubated 35 days at 8.5, 38.5 and 41.4 °C and scarified seeds incubated in 8.5 °C were null (data not presented). Once the assay was ended the seeds from 8.5 °C treatment were transferred to an optimal temperature (26 °C) in which germination attained maximum values (data not presented), showing that low temperature did not damage the embryo of the seeds. Otherwise non germinated seeds imbibed at 41.4 °C tended to exhibit deterioration signs at the end of the assays.

The observed minimum temperature for germination (T_m) lied in the 11.5 °C - 12.5 °C interval for intact seeds and in the 8.5 °C to 11.5 °C interval for scarified ones, whereas the experimental maximum

temperatures (T_M) were 36 °C < $T_M \geq$ 38.5 °C and 38.5 °C < $T_M \geq$ 41.5 °C, respectively (figure 1A). The optimum temperature range for germinability of intact and scarified seeds was 21.3 °C to 35.2 °C and 16.5 °C to 35.2 °C, respectively, showing that scarification extended the temperature interval for maximum germinability of *Cucumis anguria* seeds (figure 1A). At temperatures below 18.5 °C and above 35.2 °C the germinability of scarified seeds was higher than intact ones whereas at temperatures ranging from 18.5 °C to 35.2 °C scarification did not affect germination capacity (figure 1A). The optimum temperature interval for germination rate of both intact and scarified seeds of *Cucumis anguria* was 25.4 °C to 35.2 °C (figure 1B), coinciding with the optimal germinability. The removal of a piece of the tegument improved the germination rate only at temperatures lower than

21.3 °C and above 35.2 °C, therefore out of the optimum range (figure 1B).

The germination rates of *Cucumis anguria* seeds produced curvilinear Arrhenius plots both in scarified and intact seeds, with no abrupt changes of slope (figure 2). The curves exhibited two branches, one with negative slope in the 35.2 °C to 41.4 °C temperature interval and other with positive slope at temperatures less than 25.4 °C. Estimates of the activation enthalpies (ΔH) from closely-spaced points of the Arrhenius graph of germination rate showed that |ΔH| < 50 kJ.mol^{-1} predominate between 26.1 °C and 35.2 °C in intact seeds and between 25.4 °C and 35.2 °C in scarified ones, whereas at temperatures above 35.2 °C the limitation of the germination by processes with |ΔH| > 150 kJ.mol^{-1} is suggested (table 1). At temperatures below 25.4 °C positive ΔH was observed, with values ranging from 27.2 to 401.1 kJ.mol^{-1}, whereas at temperatures greater than 28.3 °C the rates have produced ΔH < 0 (table 1).

The analysis of the distributions of isothermal germination time of both intact and scarified seeds showed that the germination was more synchronised (lower U values) at temperatures ranging from 21.3 °C to 35.2 °C (figure 3). It can be seen that intervals for optimal germinability, germination rate and synchronisation of the germination differ to each other although they coincide in the interval from 25.4 °C to 35.2 °C.
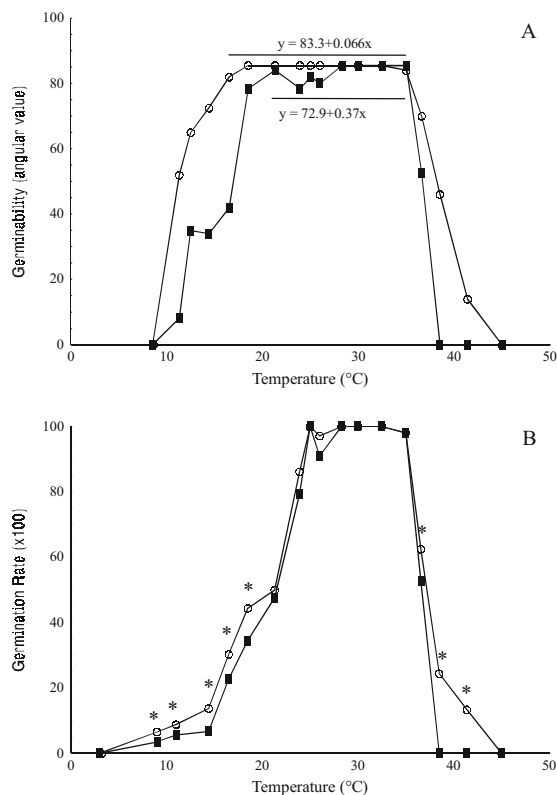

Figure 1. Temperature dependence of the germinability (A) and germination rate (B) of intact (■) and scarified (○) seeds of *Cucumis anguria*. Regression line of the optimal germinability data from intact (lower line) and scarified (upper line) seeds are shown. Asterisks show significant differences between intact and scarified seeds (Wilcoxon test).
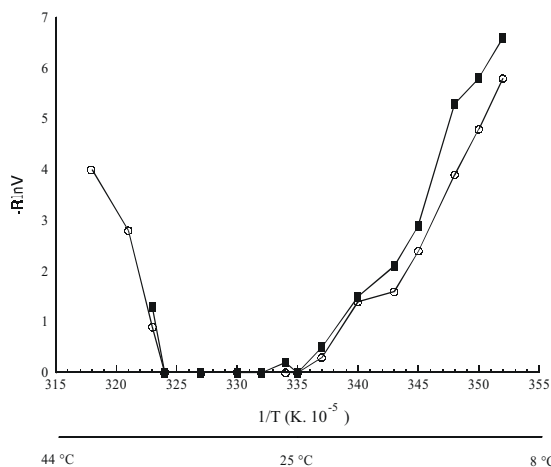

Figure 2. Arrhenius plot of the germination rate of intact (■) and scarified (○) seeds of *Cucumis anguria*.

Table 1. Activation enthalpies (ΔH) of the isothermal germination of intact and scarified seeds of *Cucumis anguria*. ΔH values were estimated from the slopes of the Arrhenius plot computed between two sucessive and close temperatures.

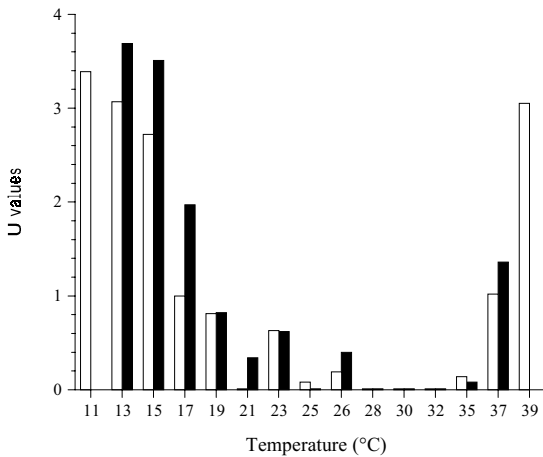| Temperature interval (°C)	Activation enthalpies (kJ.mol^{-1})	
	Intact	Scarified
11.3 - 12.5	+ 258.6	+ 268.2
12.6 - 14.4	+ 60.5	+ 153.9
14.5 - 16.5	+ 401.1	+ 258.6
16.6 - 18.5	+ 143.4	+ 131.5
18.6 - 21.3	+ 79.4	+ 27.2
21.4 - 23.9	+ 139.9	+ 149.4
24.0 - 25.4	+ 105.8	+ 69.6
25.5 - 26.1	- 93.2	- 33.0
26.2 - 28.3	+ 26.3	+ 3.9
28.4 - 30.2	- 2.7	- 2.7
30.3 - 32.5	- 2.7	- 2.7
32.6 - 35.2	- 5.5	- 5.5
35.3 - 36.6	- 355.2	- 257.8
36.7 - 38.5	-	- 399.7
38.6 - 41.4	-	- 172.2

Figure 3. Synchronism index (uncertainty, U), in bits, of the isothermal germination of intact (dark bars) and scarified (white bars) *Cucumis anguria* seeds. Germination of intact seeds at 11.3 and 38.5 °C were null.

Discussion

The optimum temperature interval for germination rate of both intact and scarified seeds was 25 °C to 35.5 °C, which overlap the range of maximum germination percentage (16 °C to 35.5 °C). Labouriau & Agudo (1987) reported that the optimum ranges of germinability and germination rate of *Salvia*

hispanica seeds did not intersect since the upper limits found were 15 °C to 25.7 °C and 28.1 °C to 32.3 °C, respectively.

Several reports have shown that the Arrhenius plot of a physiological rate process such as the seed germination is not linear (Perez & Moraes 1990, Zpevak & Perez 1993, Cardoso 1999). Labouriau & Labouriau (1991) demonstrated that when the whole temperature range of a physiological process is taken into account, the Arrhenius plot is necessarily curvilinear, with a minimum at the optimal range of the rate. Thus, if one considers the slope of the Arrhenius plot as the activation energy (E_a) of the process, E_a of rate processes with cardinal temperatures is temperature dependent. Since E_a = ΔH + RT (Labouriau & Labouriau 1997), and since the RT term cannot account for the large variation of slopes in Arrhenius plots only, these authors have suggested a temperature dependence of ΔH of such processes, as seed germination. Oliveberg *et al.* (1995) examined the temperature dependence on the refolding rate of two purified chymotrypsin inhibitors and concluded that temperature dependence of ΔH is due to a difference in heat capacity between the denatured ground state and the activation barrier. Furthermore, these authors have shown that the protein-folding process can exhibit a negative activation enthalpy.

The isothermal germination of *Cucumis anguria* seeds presented ΔH < 0 values above 28.3 °C and ΔH > 0 below 25.4 °C (table 1). According to Labouriau & Labouriau (1997) the sign of ΔH indicates if the whole physiological process is limited by partial processes whose synergistic effects are increased by raising temperatures (ΔH > 0) or it is limited by processes with antagonistic effects, i.e., partial processes not coupled to embryo growth, promoted by raising temperatures (ΔH < 0). Cardoso (1999) reported the occurrence of negative ΔH values in *Catharanthus roseus* at temperatures above 29.5 °C.

The occurrence of |ΔH| < 50 kJ.mol^{-1} between 26.1 °C and 35.2 °C in intact seeds suggests that germination of *Cucumis anguria* may be limited by diffusion processes at that temperature range. Such a temperature range is slightly broader in the scarified batch, suggesting that diffusion processes may limit the germination at the 25.4 °C - 35.2 °C interval. These ranges are close to that obtained to *Lycopersicon esculentum* (Labouriau & Osborn 1984), *Trifolium*

platense (Hageseth & Young 1994) and *Prosopis juliflora* (Perez & Moraes 1990), respectively 25.9 °C to 29.5 °C, 20.2 °C to 28 °C and 25 °C to 35 °C, within which the germination may be controlled by diffusion processes. Several researchers have shown that diffusion processes are rate-limiting in the optimum thermal range, as observed in *Sida glaziovii* (Cardoso 1992) and *Vicia graminea* (Labouriau 1970). In those seeds, the scarification of the seed coat and the consequent elimination of a barrier to the water diffusion influences the germination only in the optimum range, thus it is expected that treatments improving the diffusion through the seed coat can promote the germination in that range. Such an effect of the scarification was not observed in *Cucumis anguria* seeds showing that removal of the tegument do not improve this diffusion phenomena in the optimum range. These results also suggest that the seed coat do not affect *Cucumis anguria* germination by interfering with diffusion processes, since the rate limitation by such processes cannot occur near the extremes temperature range (Labouriau & Labouriau 1997).

Processes with $|\Delta H| > 125$ kJ.mol^{-1}, namely that involving thermal-denaturation of proteins and/or phase transitions of membrane lipids, can be occurring at T < 23.9 °C and T > 35.2 °C, which exhibited in general relatively high $|\Delta H|$ values (table 2). Thus, in *Cucumis anguria* the removal of a piece of the testa has promoted the germination at a temperature range in which that process appears to be limited by phenomena involving thermal-transconformation of proteins that can lead to the inactivation of enzymes. Thus, since enzymatic weakening of the seed coat may be a key event regulating the timing of radicle emergence in a number of species, as showed in *Cucumis melo* L. (Welbaum *et al.* 1998), we suggest the testa of *Cucumis anguria* can limit embryo expansion, which requires enzymatic processes to overcome the constraint. Therefore the removal of the testa can improve radicle elongation chiefly at temperatures affecting such processes.

As reported by Labouriau & Osborn (1984) the index U (uncertainty) can be used for measuring the synchronisation of the germination of individual seeds. In this study the germination of both intact and scarified *Cucumis anguria* seeds was less synchronized at temperatures below 23.9 °C and above 35.2 °C, which have exhibited larger U values (figure 3). Lima *et al.* (1997) observed in *Enterolobium*

contortisiliquum seeds a larger temporal distribution of seed germination at lower temperatures. The low synchronisation observed in this work may be associated to thermal-transconformation phenomena at temperatures close to T_m and T_M, as suggested above (table 1).

Further detailed investigations are required for revealing the processes of the *Cucumis anguria* germination, specially that related to the influence of the seed coat. The present study provides some contribution to the knowledge of the action of the testa on germination and rises some questions related to the environmental control of the seed germination. In conclusion, the analysis of activation enthalpies from isothermal germination rate data suggested that the seed coat of *C. anguria* acts by limiting embryo expansion rather than interfering with diffusion processes. It is suggested also that effect appears to involve enzymatic processes related to the weakening of the seed coat, thus allowing the embryo growth and ultimately the protrusion of the primary root.

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