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## Hydrotime model can describe the response of common bean (*Phaseolus vulgaris* L.) seeds to temperature and reduced water potential

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**ABSTRACT.** Germination parameters of the response to temperature and water potential from four common bean (*Phaseolus vulgaris*) lines based on thermal-time and hydrotime concepts were estimated to verify to what extent they can predict germination under different thermal and water conditions. The cultivars IPR Uirapuru and IAPAR 81 (drought-tolerant), and Grauna and Carioca (not tolerant) were used. The isothermal assays were performed in a temperature gradient block, and the assays with different osmotic potentials (PEG 6000) were performed in germination chambers. Seeds from drought-tolerant cultivars spent less time to germinate at supra-optimum temperatures than non-tolerant ones, and the cultivar Uirapuru (drought-tolerant) germinated faster in response to reduced  $\Psi$  and low temperatures. The parameter  $\Psi_{b(50)}$  did not discriminate between drought-tolerant and non-tolerant lines at the infra-optimum temperature range, but it can be used to identify drought-tolerant lines at high temperatures. In general, the hydrotime model reproduced the actual germination data relatively well, chiefly at higher temperatures. This study evidenced that the hydrotime model can be used to describe the germination of common bean seeds under reduced water potentials, and as a screening tool for drought-tolerant bean genotypes.

**Keywords:** Carioca, Grauna, IAPAR 81, Uirapuru, germination, model.

### O modelo 'hydrotime' pode descrever a resposta de sementes de feijão comum (*Phaseolus vulgaris* L.) à temperatura e potenciais de água reduzidos

**RESUMO.** Neste trabalho foram determinados alguns parâmetros térmicos e hídricos da germinação de quatro cultivares de feijão comum (*Phaseolus vulgaris*), com base nos modelos graus.dia (thermal-time) e psi.dia (hydrotime), testando-se sua adequação em descrever a germinação em diferentes condições de temperatura e água. Foram analisadas as cultivares IPR Uirapuru e IAPAR 81 (tolerantes à seca), e Grauna e Carioca (não-tolerantes). Os ensaios isotérmicos foram realizados em bloco termogradiente, enquanto que os experimentos com diferentes potenciais osmóticos (PEG 6000) foram realizados em câmaras de germinação. Sementes das cultivares tolerantes à seca germinaram mais rapidamente do que as não-tolerantes em temperaturas supraótimas, sendo que a cultivar Uirapuru (tolerante à seca) germinou mais rápido do que as demais em resposta ao  $\Psi$  reduzido e temperaturas baixas. O parâmetro  $\Psi_{b(50)}$  não discriminou entre cultivares tolerantes e não-tolerantes à seca na faixa térmica infraótima, embora ele possa ser usado para identificar cultivares tolerantes em temperaturas supraótimas. Em geral, o modelo *hydrotime* descreveu bem o comportamento germinativo, principalmente em temperaturas altas. Este trabalho mostra que o modelo pode ser usado para descrever a germinação de feijão comum em potenciais de água reduzidos, e como ferramenta para identificar genótipos de feijoeiro tolerantes à seca.

**Palavras-chave:** Carioca, Grauna, IAPAR 81, Uirapuru, germinação, modelo.

### Introduction

*Phaseolus vulgaris* (common bean) is the best known and most widely cultivated species of *Phaseolus*, being one of the most important protein sources in the diet of Brazilian people (MOREIRA et al., 2010). Several hundreds of cultivars are known and new cultivars adapted to environmental fluctuations, which meet the interests of consumers are introduced annually. Bean yield is greatly affected by climatic conditions (especially rainfall and temperature) prevailing during

the crop cycle. High temperatures adversely affect the flowering and fruiting, low temperatures reduce production and water stress affect bean plants particularly during flowering and early stages of pod formation (SILVA; COSTA, 2003). In recent years the exploration of the potential interspecific crosses within the genus *Phaseolus* has attracted the attention of breeders, and the first step towards the knowledge of these species is the characterization and evaluation lines or accesses available in the collections of bean germplasm (SILVA; COSTA, 2003).

Seed germination is one of the most vulnerable stages of the plant life cycle. The germination includes a sequence of processes through which the seeds integrate the signals from their environment and initiate radicle growth. That critical 'decision' of the seed on germinate influences the likelihood of seedling survival, which depends on suitable conditions of water, temperature, light and nutrients for subsequent seedling growth (BRADFORD, 1997). In agriculture, the seed germination and seedling emergence determine uniformity and crop stand density, as well as the yield and quality of the crop. For example, low temperatures at sowing delay both germination and emergence of common bean, leading to a lengthened crop cycle and increasing production costs due to a greater irrigation time (OTUBO et al., 1996). Thus, knowledge of the physiological requirements of the seeds and their physical interrelationships with the environment is important in ensuring successful seed germination and stand establishment (HADAS, 2004).

Temperature and water availability are the main environmental factors that influences germination and subsequent seedling growth. Models that describe seed germination should take into account the time course of seed germination, germination rate and germinability as affected by external factors singly or in combination. Moreover it is desirable that model parameters be quantifiable, have biological meaning and be based on measured germination patterns (HADAS, 2004). In line with that, threshold models as that based on thermal-time concept can properly describe the range of responses from a seed population to constant environmental conditions, being reasonably successful in predicting germination under variable temperature, sites and water potential conditions (FINCH-SAVAGE, 2004; RAWLINS et al., 2012). Ferguson et al. (2011) reported thermal-time as auxiliary tool for modeling cold hardiness for *Vitis* genotypes. The fundamental concepts of thermal-time were reviewed by Bradford (1995). In synthesis, most seeds exhibit a minimum ( $T_b$ ), an optimum ( $T_o$ ) and a maximum ( $T_c$ ) temperature for germination. A thermal time ("degrees-day" or heat sums) approach can be used to describe the germination time-course at different suboptimal ( $T_o > T \geq T_b$ ) temperatures according to (Equation 1)

$$\theta_T(g) = (T - T_b)t_g \quad (1)$$

Where:  $\theta_T$  is the thermal time to g% of the seeds complete the germination,  $T$  is the actual temperature and  $t_g$  is the time to germination of the

fraction g%. At temperatures above the optimum ( $T_o < T \leq T_c$ ), the germination rate decreases with temperature and the thermal time Equation is (Equation 2)(GARCIA-HUIDOBRO et al., 1982).

$$\theta_T = (T_c - T)t_g \quad (2)$$

The general case for non-dormant seeds is that  $T_b$  and  $\theta$  (supra-optimum) values exhibit little variation, whereas  $T_c$  and  $\theta_g$  (infra-optimum) vary with seed in a population. The thermal time model has been extended to describe the germination response to different water potentials according to the Equation (Equation 3) (GUMMERSON, 1986),

$$\theta_H = (\Psi - \Psi_{b(g)})t_g \quad (3)$$

Where:  $\theta_H$  is the "hydrotime" constant,  $\Psi_{b(g)}$  is the threshold or base  $\Psi$  below which the germination is prevented and  $\Psi$  is the actual water potential. Unlike  $T_b$  and similarly to  $T_c$ ,  $\Psi_b$  is assumed to vary among individual seeds, whereas  $\theta_H$  is constant. Within a seed population, a normal distribution of  $\Psi_b$  values among seeds would account for the differences in germination rates among seeds in the population. The hydrotime modeling approach can help predicting seed germination in soil under water deficit conditions, as reported by Patané and Tringali (2011) for *Brassica carinata* A. Braun, as well as it provided a good description of the germination time courses of *Digitaria sanguinalis* Royle at a range of salt concentrations (ZHANG et al., 2012).

Our goal was to determine some germination parameters of the response to temperature and water potential of four common bean (*Phaseolus vulgaris*) lines based on thermal-time and hydrotime concepts (FINCH-SAVAGE, 2004), and assess to what extent they can be used to predict germination under different thermal and water conditions, thus serving as potential screening tool for beans genotypes showing improved germination under a wide range of sowing conditions.

## Material and methods

Two heat-tolerant and water stress-tolerant, and two susceptible lines of common bean (*Phaseolus vulgaris* L.) were used. The cultivars IPR Uirapuru and IAPAR 81, both considered tolerant, and the cultivar IPR Grauna (susceptible) were obtained from Instituto Agronômico do Paraná (IAPAR, Londrina, Paraná State), whereas the cultivar Carioca comum (susceptible) was provided by Instituto Agronômico de Campinas (IAC,

Campinas, São Paulo State). All the seeds were harvested in November 2006 and stored in paper bags at  $\pm 7^{\circ}\text{C}$ . The viability of the seed lots were assayed monthly by tetrazolium test 0.3% (Wt/V).

The isothermal incubation was carried out on polypropylene trays lined with three qualitative filter-paper strips saturated with distilled water and placed inside closed glass tubes (250 x 25 mm) in a temperature gradient block described in details by Cardoso (2010). The block consists of ten successive vertical sets of aluminum tubes, and each set is referred to as a 'thermal station' with six replicates, one of which contained a thermal sensor and the other five received the glass tubes with seeds. Thus, every temperature station contained five glass tubes with 20 seeds per tube. The temperature in the thermal stations was measured with PT100 thermistors, connected to an electronic thermometer. The germination assays were performed in darkness at temperatures of 7, 8.5, 10.3, 11.3, 15.2, 17, 18.8, 20.2, 21.4, 22.3, 25, 26.7, 29, 30.5, 31.2, 31.7, 32.6, 33.5, 34, 34.5, 35, 35.7, 36.5, 37, 37.6, 38.2 and  $39^{\circ}\text{C}$ , with a maximum variation of  $\pm 0.2^{\circ}\text{C}$ . The germination (radicle protrusion followed by geotropic curvature) was recorded every 12h, and after each count, the germinated seeds were discarded and the glass tubes were placed randomly in the respective thermal station.

The assays with different osmotic potentials were performed in 90 mm diameter glass Petri dishes, on two layers of filter paper saturated with distilled water or solution. The dishes (five replicates with 20 seeds each) were placed in germination chambers at constant ( $\pm 0.4^{\circ}\text{C}$ ) temperature regimes of 15, 20 and  $25^{\circ}\text{C}$  (infra-optimum range), and 32, 35 and  $37.5^{\circ}\text{C}$  (supra-optimum range), in darkness. The water potential of the germination medium were controlled by solutions of polyethylene-glycol (PEG 6000) prepared according to Villela et al. (1991). The nominal water potentials of 0.0, -0.1, -0.2, -0.3, -0.4, -0.6, -0.8, -1.0 and -1.1 MPa were tested. In order to minimize the drying effect and maintain an approximate known osmotic potential the seeds were transferred every 24h to filter paper soaked with fresh PEG solutions.

The variables germinability (G%), germination rate (GR) and synchronization (U) were calculated according to Labouriau (1983). The optimum range of germinability and synchronization were found by searching for the temperature range in which the G% and U values, respectively, were highest and the regression line of the values on temperature (T) was parallel to the T axis. Germination rates were compared by stepwise exhaustive comparisons of

isotherm pairs (two tailed Wilcoxon test) (SOKAL; ROHLF, 2012), and the optimum range was that in which the temperatures produced the highest GR values and the rates of any two pairs of optimum temperatures were statistically similar.

The parameters  $\theta_T$ ,  $T_b$ ,  $T_c$ ,  $\theta_H$  and  $\Psi_b$  were estimated according to Ellis et al. (1986) and Bradford (1995). For determination of  $T_b$  and  $\theta_T$  (infra optimum), the cumulative daily germination percentages were transformed to probit and regressed against log of thermal time according to the Equation: (Equation 4),

$$\text{probit}(g) = k + \log[(T - T_b)t_g]/\sigma_{\theta} \quad (4)$$

Where:  $k$  is the intercept and  $\sigma_{\theta}$  is the standard deviation in thermal time (= inverse of the slope of the probit regression line). Repeated regression analyses were performed varying the value of  $T_b$  until the best fit (higher  $R^2$ ) was obtained (BRADFORD, 1995). The following infra-optimum temperature ranges were considered for the cultivars Carioca, IAPAR 87, Grauna and Uirapuru, respectively:  $11.3\text{--}28.9^{\circ}\text{C}$ ;  $11.3\text{--}31.7^{\circ}\text{C}$ ;  $11.3\text{--}26.7^{\circ}\text{C}$ ; and  $11.3\text{--}30.5^{\circ}\text{C}$ . For  $T_c$  estimation the Equation (Equation 5) (ELLIS et al., 1986)

$$\text{probit}(g) = k + \log[T + \theta/t_g]/\sigma \quad (5)$$

was used, where the factor  $[T + \theta/t_g]$  is equal to  $T_{c(g)}$  and  $\sigma$  is the standard deviation in  $T_c$ . To found  $T_c$  and  $\theta$ , probit (g) at supra-optimum temperatures were regressed on log  $T_c$  and different values of  $\theta$  were tried until the best fit. The supra-optimum temperature ranges for the cultivars Carioca, IAPAR 87, Grauna and Uirapuru used in the analysis were, respectively,  $35\text{--}37.6^{\circ}\text{C}$ ;  $33.5\text{--}37.6^{\circ}\text{C}$ ;  $35.7\text{--}38.2^{\circ}\text{C}$ ; and  $35.7\text{--}38.2^{\circ}\text{C}$ . The probit analysis technique referred to as above was also used to estimate the values of  $\theta_H$  and  $\Psi_b$  and  $\sigma_{\Psi_b}$  (the standard deviation of  $\Psi_b$  among seeds). For the infra-optimum temperatures the linear model was (Equation 6) (BRADFORD, 1995),

$$\text{probit}(g) = [(\Psi - \theta_H/t_g) - \Psi_{b(50)}]/\sigma_{\Psi_b} \quad (6)$$

Where:  $\Psi_{b(50)}$  is the median  $\Psi_b$  and  $\sigma_{\Psi_b}$  is the standard deviation in  $\Psi_b$  among seeds in the population. Daily cumulative germination percentages at different water potentials were combined into a single regression of probit (g) as a function of  $\Psi_{b(g)}$  ( $= \Psi - \theta_H/t_g$ ), and different values of  $\theta_H$  were used until the highest  $R^2$  was obtained. Median  $\Psi_b$  is then the midpoint of the regression

line (probit (g) = 5), and  $\sigma_{\psi_b}$  is the reciprocal of the slope. One regression was performed for each infra-optimum temperature and the  $\Psi_{b(g)}$  distributions were used to generate theoretical germination time courses at each infra-optimal T and  $\psi$  combination. For the supra-optimum temperature range the hydrotime parameters were derived by fitting the combined  $\psi$  data at each temperature using the common  $\theta_H$  obtained through the model (Equation 7) (ALVARADO; BRADFORD, 2002),

$$\text{probit}(g) = \{[\Psi - k_T(T - T_o)] - (\theta_H/t_g) - \Psi_{b(50)}\} / \sigma_{\psi_b}, \quad (7)$$

Where:  $k_T$  is the slope of the  $\Psi_{b(g)}$  versus T line when  $T > T_o$  (optimal temperature) and  $\theta_H$  is the hydrotime value at  $T_o$ . That common  $\theta_H$  value was then replaced by  $\theta_H$  in the Equation 6. The expected tg values obtained at each supra-optimal temperature were then used to produce the predicted germination curves at the different  $\Psi$  and supra-optimal T.

## Results and discussion

Preliminary assays for the seed characterization showed that the seed mass (100-seeds weight) ranged from 26.6 g ( $x \pm 1.7$  g) in the cultivar Carioca to  $23.9 \pm 0.61$  g in the Uirapuru, whereas the water percentage (fresh weight basis) of not imbibed seeds ranged from  $13.1 \pm 0.8\%$  (cv. Carioca) to  $11.2 \pm 0.4\%$  (cultivars Grauna and Uirapuru). The time for the seeds imbibe 50% in distilled water at 25°C was between 60 and 120 min. The viability of the seed lots assessed by tetrazolium test was 100%; the germinability (maximum germination capacity) at 25°C was above 90%; and the average germination rate at 25°C ranged from  $0.245 \pm 0.028$  h<sup>-1</sup> (cultivar Carioca) to  $0.325 \pm 0.017$  h<sup>-1</sup> (cultivar Uirapuru). The maximum germinability was achieved at the temperature interval of approximately 11 to 36.5°C for IAPAR 81, IPR Grauna and IPR Uirapuru, and at 13-36.5°C for Carioca seeds (Table 1). The optimum range of germination rate (GR) was 29-35 °C for the cultivars Carioca, IAPAR 81, Grauna and Uirapuru, with relatively few differences among them, with exception of the IAPAR 81 that showed a single temperature optimum (Table 1). The synchronization of the germination appraised through the U index was higher (optimal), i.e., presented the lowest U values (minimum rate heterogeneity) in a range that overlaps the optimum range of germinability for each cultivar (Table 1). Since the optimum temperature range for GR overlaps both the optimum range for

germinability and synchronization, it can be taken as optima for the germination of the cultivars under a constant temperature regime. Those optimum ranges were relatively high and narrow, compared with the values reported by Machado-Neto et al. (2006) for several races of common bean returned from the germplasm collection of the CENARGEN/EMBRAPA, IAC and IAPAR. Also the optimum temperatures were similar among the cultivars and did not allow to discriminate between heat-tolerant and non-tolerant races.

**Table 1.** Thermal parameters of the germination of *Phaseolus vulgaris*, cvs. Carioca, IAPAR 81, IPR Grauna and Uirapuru.  $G_{opt}$  = optimum temperature for germinability;  $GR_{opt}$  = optimum temperature for average germination rate;  $U_{opt}$  = optimum temperature for synchronization;  $\theta_{T(50)}$  = median thermal time;  $\sigma_{\theta_T}$  = standard deviation of  $\theta_T$ ;  $T_b$  = base temperature;  $\theta$  = thermal time (supra-optimum range of T);  $Tc_{(50)}$  = median ceiling (maximum) temperature;  $\sigma_{Tc}$  = standard deviation of Tc.

		Carioca	IAPAR 81	IPR Grauna	Uirapuru
full range	$G_{opt}$ (°C)	13-36.5	11.5-36.5	11-36	11-36.5
	$GR_{opt}$ (°C)	30.5-34.5	32.5	29-35	31-34.5
	$U_{opt}$ (°C)	15-37	25-35	25-36	20-36.5
infra-optimum range	$\theta_{T(50)}$ (° h)	589	706	667	493
	$\sigma_{\theta_T}$ (° h)	247	320	251	199
	$T_b$ (°C)	8.9	8.4	8.9	9.2
supra-optimum range	$\theta$ (° h)	103	280	110	97
	$Tc_{(50)}$ (°C)	38.5	42.3	38.6	38.9
	$\sigma_{Tc}$ (°C)	1.3	2.3	1.2	1.0

The thermal time ( $\theta_T$ ) required for 50% of the seeds to germinate were relatively similar among the cultivars, with the greater nominal differences observed between IAPAR 81 (706°h) and Uirapuru (493°h). Thus, based on the results obtained here, the cultivar IAPAR 81 required more degrees.hour for 50% of the seeds to germinate at a given sub-optimum temperature than Uirapuru. In other words, IAPAR 81 seeds spent longer time to germinate than Uirapuru ones in the infra-optimum range of T. Considering the base temperature ( $T_b$ ) and the median  $\theta_T$  ( $\theta_{T(50)}$ ) values obtained in this paper, the cultivars Carioca, IAPAR 81, Grauna and Uirapuru would spend around 96, 107, 109 and 85h, respectively, for 50% of the seeds to germinate at 15°C. On the other hand, at the supra-optimum range the thermal time model predicts that GR decreases with temperature, that different fractions of the seed population have different ceiling temperatures ( $Tc$ ), and that the slope of this decrease is the same for all the seeds in a population, that is, the total thermal time remained constant in the supra-optimal range of T whereas differences in GR among seeds were a consequence of the variation in the  $Tc$  values (ALVARADO; BRADFORD, 2002). Based on that assumption and applying the probit analysis to the germination time

courses at the supra-optimum range, we obtained median  $T_c$  values ranging from 38.5°C (Carioca) to 42.3°C (IAPAR 81) (Table 1). From the parameters  $\theta_T$  and median  $T_c$  ( $T_{c(50)}$ ), the estimated germination time (in hours) for 50% of the Carioca, IAPAR 81, Grauna and Uirapuru seeds to germinate at 37°C (for example) would be, respectively, 69, 53, 69 and 51h. Thus, the thermal time parameters suggest that seeds from cultivars considered drought and heat-tolerant spend less time to germinate at supra-optimum temperatures than non-tolerant ones.

The effects of low osmotic potential on germination were analyzed through the hydrotime parameters, listed in Table 2. At the infra-optimum temperatures of 15, 20 and 25°C the median  $\Psi$  base ( $\Psi_{b(50)}$ ) values exhibited a relatively small variation within each cultivar, whereas the hydrotime constant ( $\theta_H$ ) values decreased as temperature increased from 15 to 20°C and were similar between 20 and 25°C. The  $\theta_H$  approach assumes germination rate as a function of the difference between the actual  $\Psi$  and a threshold base  $\Psi$  ( $\Psi_b$ ) below which germination is prevented (FINCH-SAVAGE, 2004). Such differences times the 'real' time for germination of a given percentage (tg) is the  $\theta_H$ , which may account for the decrease in GR of the common bean cultivars as temperature is reduced from 20 to 15°C. In other words, as  $T$  decrease in the infra-optimum range, the seeds require more accumulated hydrotime and, consequently, they take longer to germinate. In the *P. vulgaris* lines tested here the increase in  $\theta_H$  with decreasing  $T$  appears to be less conspicuous as temperature approaches the optimum range, as suggested by the relatively small differences in  $\theta_H$  between 20 and 25°C. Comparing the cultivars to each other and considering that the  $\theta_H$  indicate the overall germination rate

(BRADFORD; STILL, 2004) – the lower the  $\theta_H$  is, the shorter the time required to a given seed fraction to germinate – the cultivar Uirapuru germinated faster than others in response to reduced  $\Psi$  and relatively low temperatures.

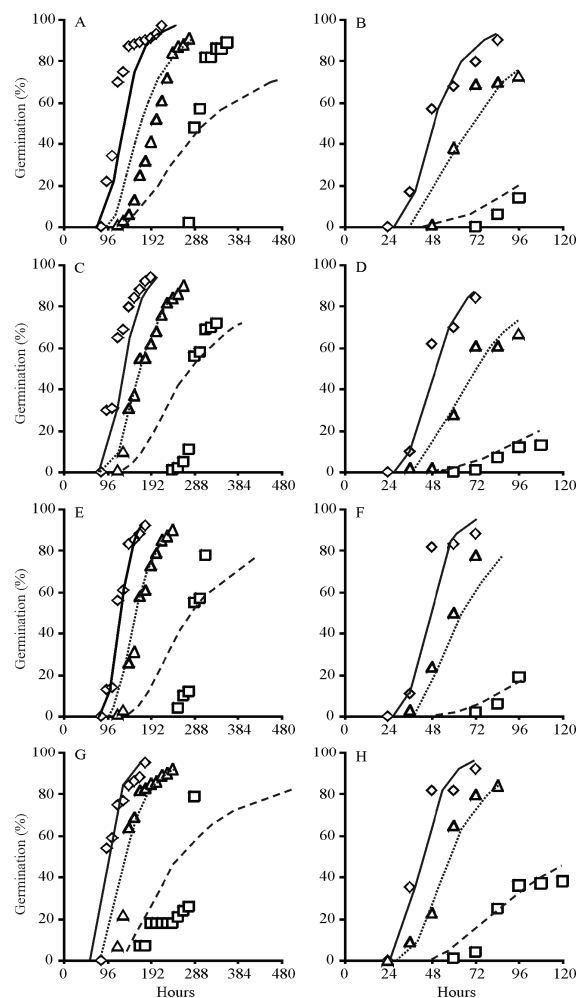
$\Psi_{b(50)}$  values were obtained for the different cultivars, both at low (infra-optimum) and high (optimal and supra-optimal) temperatures. When the values corresponding to each cultivar were arranged in ascending order (from more negative to less negative  $\Psi_b$ ) it was observed that the relative ranking of the cultivars in terms of  $\Psi_{b(50)}$  changed with  $T$  in the infra-optimum range. Thus, IAPAR 81 – a moderate drought-tolerant cultivar (IAPAR, 2008) – presented the lowest  $\Psi_{b(50)}$  (-1.02 MPa) at 15°C, whereas the cultivar Carioca (non-tolerant) exhibited the highest value (-0.85 MPa) (Table 2). Otherwise, at 20 and 25°C the lowest and the highest  $\Psi_{b(50)}$  values were found for the cultivars Carioca and Grauna, respectively. The  $\Psi_{b(50)}$  value of a seed population indicates its inherent stress tolerance as well as it can be considered as a general vigor index (BRADFORD; STILL, 2004). The more negative the  $\Psi_{b(50)}$  is, the lower  $\Psi$  required to prevent germination (see Equation 3) and the more rapid germination will be at a given  $\Psi$  whose value is greater than seed's  $\Psi_b$ . Thus, the relative response in terms of water stress tolerance of the cultivars tested here varied with  $T$  in the infra optimum range, in which  $\Psi_{b(50)}$  did not discriminate well between drought-tolerant and non-tolerant *Phaseolus vulgaris* lines, whose selection is based on their sensitivity to water stress during the reproductive phase from flowering to the pod formation (MORAES et al., 2010).

**Table 2.** Hydrotime parameters of the germination of *Phaseolus vulgaris*, cvs. Carioca, IAPAR 81, IPR Grauna and Uirapuru, under different temperatures.  $\theta_H$  = hydrotime constant;  $\Psi_{b(50)}$  = median base water potential;  $\sigma_{\Psi_b}$  = standard deviation of  $\Psi_b$ .

		Carioca	IAPAR 81	IPR Grauna	Uirapuru
15°C	$\theta_H$ ( $\Psi$ , h)	84	117	110	82
	$\Psi_{b(50)}$ (MPa)	-0.85	-1.02	-0.99	-0.92
	$\sigma_{\Psi_b}$ (MPa)	0.26	0.22	0.17	0.22
20°C	$\theta_H$ ( $\Psi$ , h)	62	53	41	40
	$\Psi_{b(50)}$ (MPa)	-0.92	-0.78	-0.71	-0.79
	$\sigma_{\Psi_b}$ (MPa)	0.29	0.28	0.30	0.27
25°C	$\theta_H$ ( $\Psi$ , h)	58	53	43	37
	$\Psi_{b(50)}$ (MPa)	-0.91	-0.83	-0.80	-0.89
	$\sigma_{\Psi_b}$ (MPa)	0.26	0.28	0.31	0.32
32°C	$\theta_H$ ( $\Psi$ , h)	25	25	23	24
	$\Psi_{b(50)}$ (MPa)	-0.76	-0.84	-0.73	-0.87
	$\sigma_{\Psi_b}$ (MPa)	0.21	0.27	0.26	0.29
35°C	$\theta_H$ ( $\Psi$ , h)	25	25	23	24
	$\Psi_{b(50)}$ (MPa)	-0.69	-0.65	-0.67	-0.74
	$\sigma_{\Psi_b}$ (MPa)	0.20	0.19	0.16	0.17
37.5°C	$\theta_H$ ( $\Psi$ , h)	25	25	23	24
	$\Psi_{b(50)}$ (MPa)	-0.46	-0.48	-0.34	-0.49
	$\sigma_{\Psi_b}$ (MPa)	0.25	0.24	0.29	0.28

In the optimal and supra-optimal ranges of  $T$ ,  $\Psi_{b(50)}$  values became less negative with  $T$  for all the cultivars, which can account for the reduced germination rate with  $T$  in the supra-optimal range, as postulated by Alvarado and Bradford (2002). In the case of bean plants, a decrease of the germination rate may indicate a decrease in the plant growth rate (CUSTODIO et al., 2009). When comparing the cultivars, it was observed that Uirapuru (drought-resistant), presented the lowest  $\Psi_{b(50)}$  values at 32, 35 and 37.5°C. At 37.5°C, the only common supra-optimal  $T$  for all the cultivars tested here, Uirapuru and IAPAR 81 presented the lowest  $\Psi_{b(50)}$  values, suggesting that at high temperatures the  $\Psi_{b(50)}$  parameter can be used to discriminate drought-tolerant *Phaseolus vulgaris* lines. This result corroborates Patanè and Tringali (2011), according to which low base water potential along with a high germination rate are criteria of cultivar selection in breeding programs for drought tolerance.

By plotting the germination time-courses at three reduced  $\Psi$  (-0.1, -0.3 and -0.6 MPa) either at a infra-optimal (15°C) or a 'high' (35°C) temperature, revealed a delay and, in some cases, also a reduction in final germination (Figure 1). The temperature of 35°C was at the boundary between the optimal and the supra-optimal range of  $T$  for the cultivars Carioca, Grauna and Uirapuru, and it was supra-optimal for IAPAR 81. At 15°C, the hydrotime model (curves) reproduced the actual germination data (symbols) relatively well at -0.1 MPa and -0.3 MPa but not at -0.6 MPa, where the differences between observed and expected germination times were significant ( $p < 0.05$ , Chi-Square). At 35°C, the predicted germination times by the hydrotime model closely matched actual germination time-courses at -0.1 to -0.6 MPa PEG solutions. The deviate from expectations observed for the treatments of 15°C combined with -0.6 MPa may indicate, as pointed by Bradford (1997), an alteration in physiology such that the germination delay at lower  $\Psi$  cannot be explained in terms of reduction of hydrotime accumulation rate. The use of the thermal-time model to describe the time-courses of the common beans isothermal germination (MACHADO-NETO et al., 2006) was extended in this paper to include the effect of the water potential. Otherwise, thermal and hydrotime germination parameters can differ from hydrotime growth parameters, as shown by Ravenau et al. (2011) who suggested that germination and early common bean seedling growth 'need to be analyzed separately in breeding programs to improve emergence rates'.



**Figure 1.** Germination time courses of *Phaseolus vulgaris* cv. Carioca (A, B), cv. IAPAR 81 (C, D), cv. Grauna (E, F) and cv. Uirapuru (G, H) at 15°C (left column) and 35°C (right column), and -0.1 MPa ( $\diamond$ ), -0.3 MPa ( $\Delta$ ) and -0.6 MPa ( $\square$ ). The symbols are the actual data, and the curves are the time courses predicted by the hydrotime model for seeds germinated at -0.1 MPa (solid line), -0.3 MPa (dotted line) and -0.6 MPa (dashed line). The parameters values are shown in Table 2.

## Conclusion

The thermal time parameters suggested that seeds from cultivars considered drought and heat-tolerant germinated faster than non-tolerant ones under supra-optimum temperatures. Furthermore, at supra-optimal temperatures  $\Psi_{b(50)}$  can be used to discriminate drought-tolerant *Phaseolus vulgaris* lines, e.g. cultivar Uirapuru. This study evidenced that the hydrotime model can also be effective in describing the isothermal germination of common bean seeds under relatively low water potentials (-0.4 MPa  $\cong$  -3.9 atm) at different temperatures.

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