Scientific Paper

Stimulating effect of thermotherapeutic treatments on seeds of *Glycine max* L.

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Abstract

Objective: To evaluate the effect of the stimulation of diverse thermotherapeutic treatments, based on dry heat, on seeds of nine cultivars of *Glycine max* L., at three temperatures and three exposure times.

Materials and Methods: The study was conducted in the laboratories of the Department of Plant Genetics and Breeding, of the National Institute of Agricultural Sciences. The effect of the stimulation of diverse thermotherapeutic treatments on the variables germination capacity, germination rate and radicle length was determined at 48, 72 and 96 h after the seeds were put to germinate. Then, the effect on the dry biomass of 100 radicles/treatment at 120 h was found. Variance analysis and Duncan's multiple range test were performed on the variables for determining differences among their means.

Results: The most effective thermotherapeutic treatment for all the variables was the one with five hours of exposure to 50 ºC, which increased the germination rate and capacity, radicle length and dry biomass of 100 radicles compared with the control. The cultivars of the best response to the treatment of 50 ºC during five hours, regarding germination percentage, were INCASoy-24 and INCASoy-27. Regarding radicle length and mass of 100 radicles, the cultivar INCASoy-2 was the best.

Conclusions: The thermotherapeutic treatment with dry heat, which was more effective regarding the variables germination rate and capacity, and their vigor-related qualities, was the one with five hours of exposure to 50 ºC, for the nine cultivars used in the study. In the variables radicle length and dry mass of 100 radicles, the positive effect of the pregermination treatment of five hours of exposure to 50 ºC with regards to the control was proven.

Keywords: heat, stimulants, germination

Introduction

Glycine max L. (soybean) is the oil plant of highest importance in the world, and is one of the 10 most widely used crops for their great diversity of uses. This value is also consequence of investment on research, especially for obtaining information that increases productivity (Santos, 2016). For a higher yield per area, besides adequate cultural techniques, the use of high-quality seeds is essential, which is expressed in the genetic, physical, physiological and sanitary traits, which are capable of influencing the capacity to originate high-productivity plants (Medeiros *et al*., 2019). Several factors are responsible for the loss of seed quality, mainly biotic and physiological factors (Fernández-Mayer, 2018). At present, more than 25 % of the grains produced in the world are contaminated, by one or several fungi, or need to increase their stimuli in the germination process (FAUBA, 2016).

Researchers of the School of Agronomy, of the University of Buenos Aires, developed an innovative technology along with the Venado Tuerto Regional School, of the National Technological University. From a physical method, this technology would allow to improve the germination capacity and vigor of seeds from soybean and other crops. The experts emphasized that this tool is environment-friendly, and anticipated that it could begin to be implemented. They also stated that from a physical method the germination capacity and vigor of the seeds from soybean and other crops can be improved (Schneider *et al*., 2015).

The elimination or reduction of microorganisms has been efficiently achieved through chemical treatments. Yet, the search for alternative methods to stimulate seed development has gained attention in the world, because they cause less damage to the environment and to human health, mainly those treatments based on plant extracts,

Received: 03/04/2022
Accepted: 07/07/2022

How to cite a paper: Mederos-Ramírez, Alejandro & Ortiz-Pérez, Rodobaldo. Stimulating effect of thermotherapeutic treatments on seeds of *Glycine max* L.). *Pastos y Forrajes*. 45:eI21, 2022

essential oils, biological control and physical treatment (Cóbar-Carranza *et al*., 2015).

It has been proven that thermotherapy is an efficacious, chemicals-free and easily applied method to achieve stimulation of germination and seedling development. Taking the above-stated facts into consideration, the objective of this work was to evaluate the effect of the stimulation of diverse thermotherapeutic treatments, based on dry heat, on seeds from nine *G. max* cultivars, at three different temperatures, in three exposure times.

Materials and Methods

Location. The experimental essays were conducted in the laboratories of the Department of Plant Genetics and Breeding in the National Institute of Animal Science (INCA, for its initials in Spanish), between March and April, 2020.

Plant material. Original, certificated seeds, from nine commercial cultivars obtained and preserved at INCA were used: INCASoy-1, INCASoy-2, INCASoy-24, INCASoy-27, INCASoy-35 and INCASoy-36. Original and certificated seeds from three transgenic cultivars, from the Center of Genetic Engineering and Biotechnology (varieties SCIGB L-1, Carolina RP-5 and CEB-2) and preserved there, were also used.

Seeds were used that showed values of 10 to 11 % of moisture, with satisfactory health, related to their category of original. The grains were previously analyzed, which expressed satisfactory germination and development of normal seedlings, free from pathogen fungi.

Experimental design and treatments. A complete randomized design was used. Samples of 50 grains/treatment were randomly taken, with two replicas (100 grains/cultivar), for a total of 90 treatments (9 cultivars x 3 exposure times x 3 temperature ranges and the control with untreated seeds). The exposure times were: 1, 5 and 10 h and temperatures of 45, 47 and 50 ºC (maximum permissible limit for seed germination by protein denaturation).

Experimental procedure. The seeds were put in paper bags identified by cultivar, time, temperature and replica, to expose the seeds to heat in Boxun stove (design of new oven type of bgz II series, elaborated and commercialized by Co.LTD, Shanghai). Regarding the stove, the AC feeding source was 220 V ± 10 % / 50 Hz ± 2 %, temperature range + 5 ∼ 250 °C, forced circulation and high accuracy in the temperature control ± 0,5 ºC, with capacity of 1100W and work chamber 450 × 400 × 450 mm (L × D × H).

After each thermal treatment, the seeds were left to rest 24 h, at room temperature. They were put to germinate on aluminum trays. Besides the untreated control, they were covered with filter paper sheets and moisture was maintained during 72 h, with distilled water.

Evaluated variables. Variables related to germination were analyzed, as main criterion for the evaluation of the effect of the treatments on seed qualities and the vigor initially developed by the seedlings. That is: seed germination (%) at 48, 72 and 96 h, germination rate in hours (relation between germination and time), radicle length (mm) at 48, 72 and 96 h after putting the seeds to germinate. At 120 h, the effect on the dry mass of 100 radicles/treatment (dried 24 h at 80 ºC) was determined, measured in analytical scale calibrated in mg.

Statistical analysis. The variables related to the germination and radicle length were subject to factorial analysis. For the vigor expressed in germination rate, germination percentage and mass of 100 radicles, simple ANOVA analysis was carried out. The means of the treatments and the control were compared by Duncan's test ($p \leq 0,05$) using the statistical package IBM SPSS, Version 22.

Before carrying out the statistical analysis, the normality test was performed on all the evaluated variables with the equation $(j\text{-}0,5)/n = Zj$, as well as the variance homogeneity through Bartlett's test. The data normality was proven, for which the analyses were done without transforming the values.

Results and Discussion

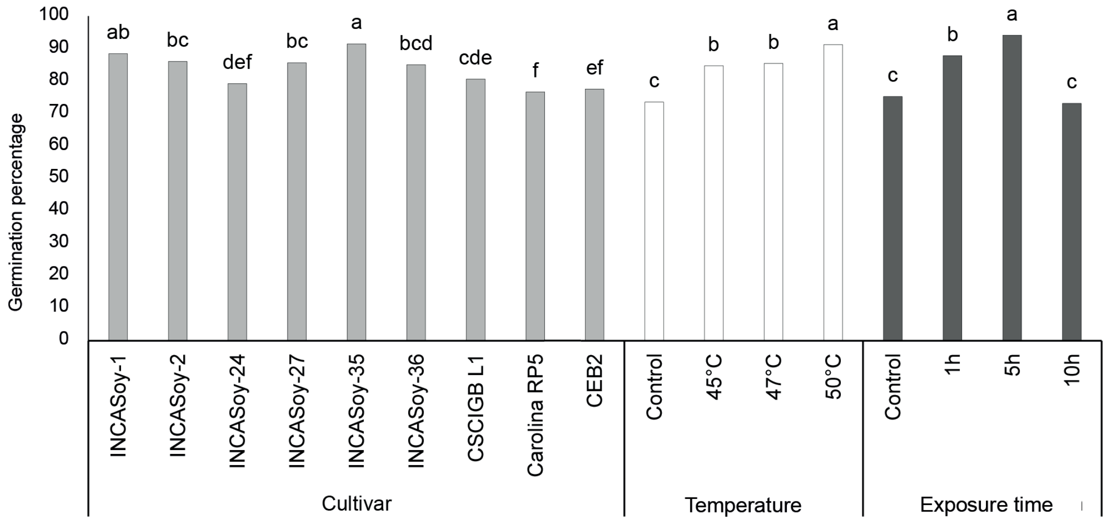
Table 1 shows the interactive effect of the factors for the variable germination percentage at 96 h. The factors cultivar, temperature and exposure time showed highly significant levels and high proportions of the total corrected quadratic mean. There was significant interaction among them, but with low proportions of the total corrected quadratic mean.

Figure 1 shows satisfactory germination percentages in all the cultivars. The genotypes INCAsoy-35 and INCAsoy-1 showed the highest germination percentages, with 91,4 and 88,4 %, respectively. The temperature of 50 ºC was the most adequate to stimulate germination, improving germination by more than 11 % with regards to the control. High temperatures can influence positively germination in some species. Varés-Megino *et al.*

Table 1. Trifactorial analysis for the variable germination percentage at 96 h after putting the seeds to germinate.

Origin	Sum of type III squares	gl	Quadratic mean	F	Sig.	Proportion MC factor/ MCTC
Corrected model	26 927,644[a]	89	302,558	12,113	0,000	
Intersection	1 099 020,062	1	1 099 020,062	43 999,913	0,000	
Cultivar	3 128,769	8	391,096	15,658	0,000	2,399
Temperature	1 164,494	2	582,247	23,311	0,000	3,572
Time	12 800,346	2	6 400,173	256,235	0,000	39,267
Cultivar x temperature	2 089,728	16	130,608	5,229	0,000	0,801
Cultivar x time	2 229,877	16	139,367	5,580	0,000	0,855
Temperature x time	681,136	4	170,284	6,817	0,000	1,045
Cultivar x temperature x time	2 495,309	32	77,978	3,122	0,000	0,478
Error	2 248,000	90	24,978			
Total	1 297 912,000	180	7 219,62			
Corrected total	29 175,644	179	162,99			

R^2 = 0,923 and corrected R^2 = 0,847



Bars with equal letters in each treatment do not differ significantly, according to Duncan's test for p= 0,05.
Figure 1. Effect of cultivar, temperature and exposure time on the germination percentage.

(2009) found that the high temperatures did not show negative effects on the germination of *Pinus contorta* Douglas, but they did on *Araucaria araucana* (Molina) K. Koch.

Regarding the exposure time, at 5 h of exposure the best response appeared, with highly significant differences.

Table 2 shows the seed germination of the *G. max* cultivars at 48, 72 and 96 h after being put to germinate, they were later subject to 50 ºC and to three exposure times.

It is evident that the treatment of five h of exposure to 50 ºC was the most effective, at 48, 72 and 96 h. At 48 h, it could surpass the control by 19,1 %, at 72 h by 15,7 % and at 96 h by 11,3 %. The treatment of 5 h at 50 ºC showed the mean germination values of the nine genotypes, higher than the controls, at all evaluation moments.

For a certain range of high temperatures, the rate of water absorption and of chemical reactions is higher, and the seeds germinate faster and without difficulty (Roca, 2018). These results could also respond to the characteristics of the seeds. Similar studies, conducted on beans, with dry heat, at high temperatures, revealed that such treatments had a positive effect on germination (Françoso and Barbedo, 2014).

The increase of the exposure times to 10 h decreased germination percentage. Similar results were obtained in the species *Vicia faba* L. Here, the highest exposure times at 55 ºC had a negative effect on germination. This was due to the inhibition of the enzymatic activity, respiratory metabolism, growth and cell division (Carvajal, 2012; Françoso and Barbedo, 2014).

The treatment of 5 h of exposure to 50 ºC was the one with the best average of accumulated germination (figure 4) and surpassed the control and the other treatments at all the evaluation moments. These results are similar to the ones reported by Ramos-Rodríguez (2021), who indicated higher accumulated average of seed germination in the species *Prosopis pallida,* subject to 60 °C.

Figure 3 shows that the treatment of 5 h of exposure to 50 ºC in the first 48 h almost doubled germination rate compared with the control, without treatment. This performance, to a lesser extent, was maintained until 96 h. That is: there was positive influence on the germination rate, which means higher potential for the adaptation to the land conditions. Similar results were reported by Jurado and Westoby (1992) and Maguire (2020), who stated that high germinability was associated to the fast germination rate; while low germinability had moderate and low germination rates.

The results related to radicle length were similar to the factorial analysis carried out for the germination percentage (figure 4). There were significant differences among the factors and their interactions. Regarding the cultivars, the genotypes INCASoy -2 and INCASoy-35 stood out. The temperature of 50 ºC improved the root length of the *G. max* cultivars by 28 mm more than the control. Likewise, the most adequate exposure time to stimulate radicle elongation was 5 h, with higher results than the control. Such treatment exceeded the controls by 37 % of germination, which proved higher germination rate (1,90 times).

The treatments of 5 h of exposure to 50 ºC showed higher values than the ones reached by the control at the three evaluation moments for all the genotypes (table 3). The treatment of 5 h at 50 ºC stood out significantly, which recorded a mean of 33,6 mm of radicle length, doubling the length of the control and significantly differing from the others.

The highest exposure time to which the seeds were subject (10 h) showed equal or lower values than the control. This response could have been influenced by a decrease of the endogenous levels

Table 2. Seed germination (%) at 48, 72 and 96 h of being put to germinate, after being subject to 50 °C and three exposure times.

Cultivar	48 h				72 h				96 h			
	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C
INCASoy-1	39,0	57,0	82,0	40,0	53,0	80,0	94,0	61,0	77,0	87,0	98,0	84,0
INCASoy-2	46,0	55,0	91,0	15,0	58,0	69,0	95,0	41,0	77,0	93,0	99,0	72,0
INCASoy-24	56,0	62,0	83,0	69,0	72,0	84,0	89,0	77,0	78,0	90,0	100,0	84,0
INCASoy-27	62,0	68,0	95,0	25,0	70,0	84,0	100,0	51,0	79,0	95,0	100,0	80,0
INCASoy-35	47,0	53,0	87,0	70,0	64,0	70,0	93,0	81,0	81,0	78,0	94,0	87,0
INCASoy-36	42,0	67,0	81,0	47,0	61,0	88,0	98,0	66,0	73,0	93,0	100,0	88,0
CIGB L-1	51,0	71,0	86,0	60,0	60,0	88,0	95,0	74,0	70,0	93,0	98,0	79,0
Carolina RP-5	37,0	51,0	79,0	48,0	56,0	71,0	86,0	62,0	67,0	88,0	94,0	69,0
CEB-2	41,0	68,0	81,0	59,0	63,0	86,0	89,0	68,0	69,0	89,0	93,0	80,0
Mean value	$46,8^{c}$	$61,3^{b}$	$85,0^{a}$	$48,1^{c}$	$61,9^{cd}$	$80,0^{b}$	$93,2^{a}$	$64,6^{c}$	$74,6^{d}$	$89,6^{b}$	$97,3^{a}$	$80,3^{c}$
VC %	14,30				2,95				1,99			
SE ±	0,2869				0,8691				0,8503			

Different letters in the same row do not differ significantly, according to Duncan's test for p = 0,05.

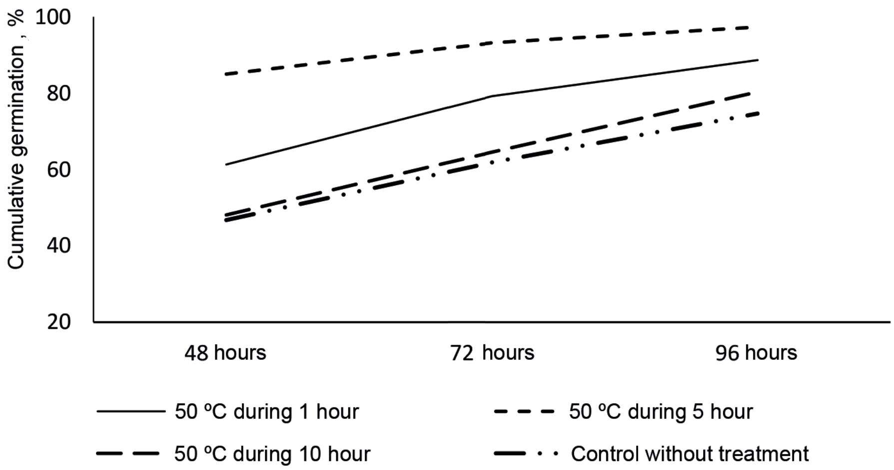
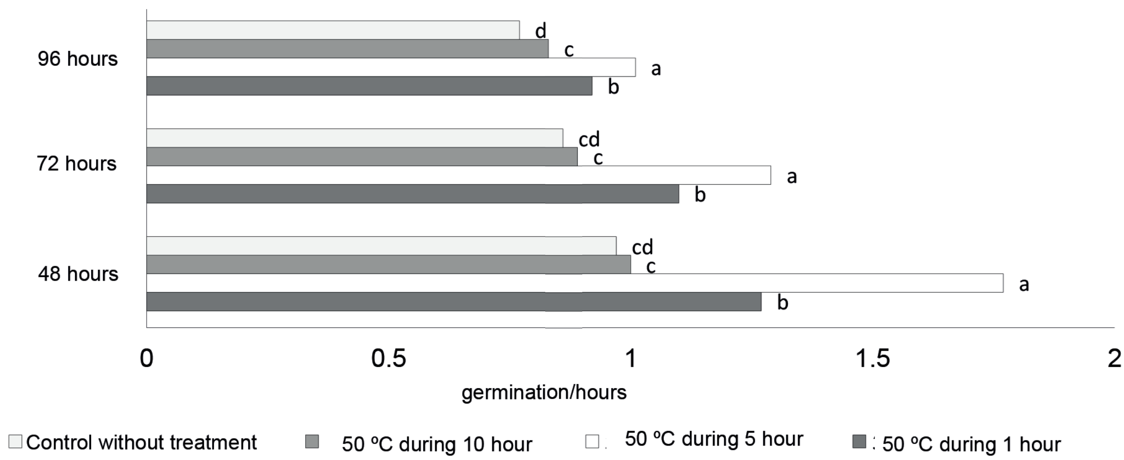


Figure 2. Accumulated average seed germination per days for the different treatments.



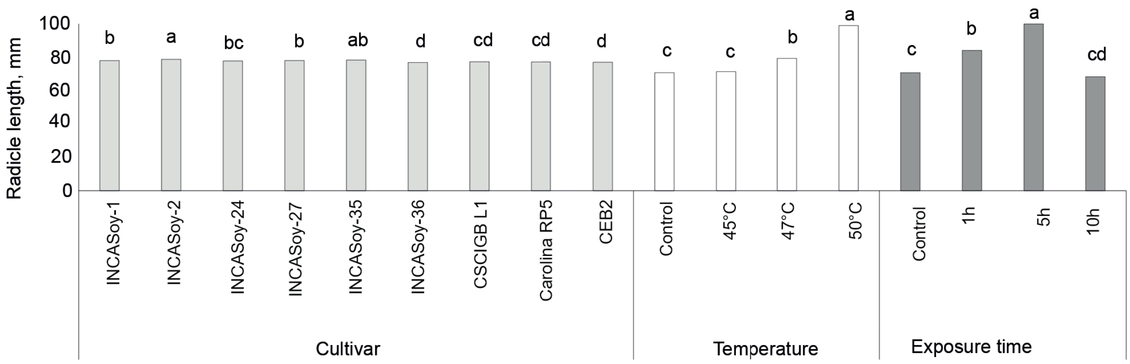
Bars with equal letters in each treatment do not differ significantly, according to Duncan's test for p = 0,05.

Figure 3. Effect of the treatments on the seed germination rate.

of growth regulators, such as cytokinins and auxins, which propitiated a decrease of growth and, thus, a reduction in radicle length. Prolonged exposure times at high temperatures influence the increase of the content of abscicic acid (ABA), which is an inhibitor, and with it the oxidative systems that reduce the level of free auxin and cause decrease of cell elongation, are activated (Torres *et al*., 2019). In addition, the effect could also be related to the dehydration of embryo tissues, caused by the prolonged thermal treatments on the seed, considerably affecting the permeability of cell membranes in the root (Zahra, 2011).

Another vigor-related quality that was evaluated at 120 h, after the seeds were subject to three exposure times at 50 ºC, was dry mass (mg) of 100 radicles/treatment. Table 4 shows that the treatment of 5 h of exposure to 50 ºC was the combination of time and most adequate temperature to promote the root development of the future seedlings from the seeds of the cultivars. Such procedure recorded an average dry mass of 108 mg, higher results than those of the control, in which an average mass of 46 mg was achieved, that is, it exceeded by 62 mg in 100 radicles.

The dry mass of 100 radicles in the exposure time of 10 h at high temperatures showed lower results than those of the control, because as has been discussed these indicators could be affected by the inhibition of the metabolic processes involved in cell division and elongation due to the exposure of the seed to high temperatures during prolonged times (Vázquez and Torres, 2006; Argentel-Martínez *et al.,* 2017). This did not occur when the seed was



Bars with equal letters in each treatment do not differ significantly, according to Duncan's test for *p* = 0,05.
Fig. 4. Effect of cultivar, temperature and exposure time on radicle length.

Table 3. Radicle length at 48, 72 and 96 h of being put to germinate, after being subject to 50 °C and three exposure times.

Cultivar	48 h				72 h				96 h			
	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C
INCASoy-1	56,8	58,2	79,4	39,1	65,4	92,7	109,1	49,9	70,9	101,7	135,8	59,0
INCASoy-2	64,2	65,1	82,7	41,3	67,2	93,9	116,2	50,8	70,3	102,7	137,7	60,3
INCASoy-24	58,4	55,2	83,9	40,6	66,6	92,5	111,4	52,2	72,0	102,2	135,0	60,7
INCASoy-27	61,7	60,1	81,4	41,3	67,2	92,2	108,4	50,9	72,7	100,0	135,8	60,0
INCASoy-35	60,2	59,2	82,1	40,7	65,3	91,9	115,0	51,8	79,1	100,7	137.0	61,4
INCASoy-36	57,9	60,0	80,6	40,4	63,2	92,0	114,2	49,2	75,3	99,6	134,9	59,2
CIGB L-1	63,2	62,3	81,6	41,0	67,4	92,2	113,5	49,2	70,2	100,0	136,2	58,7
Carolina RP-5	61,4	57,2	83,2	38,8	68,2	91,6	114,7	49,9	77,4	100,7	135,4	60,3
CEB-2	56,1	62,1	80,5	38,7	64,2	91,5	111,1	50,7	81,4	99,1	135,3	60,4
Mean value	60,5^{b}	59,7^{b}	81,7^{a}	40,4^{c}	66,3^{c}	92,3^{b}	112,8^{a}	50,5^{d}	74,3^{c}	100,9^{b}	136,0^{a}	59,9^{d}
VC %	0,53					0,67				1,85		
SE ±	0,9137					0,7533				0,6422		

Equal letters in the same row do not differ significantly, according to Duncan's test for p = 0,05.

exposed to lower times. In this case, the metabolic processes are stimulated by the increase of the water absorption rate, respiration and chemical reactions that participate in cell development, which facilitates higher rooting of the plants (Carvajal, 2018).

Thermotherapeutic treatments are the ones that mark the difference, if reaching better crop production is the aim (Alvarez and Ceballos, 2017).

Conclusions

The thermotherapeutic treatment with dry heat, more effective regarding the variables germination rate and capacity, as well as the vigor-related qualities, was the one of 5 h of exposure to 50 ºC for all the cultivars.

The cultivars with the best response to the treatment of 50 ºC during 5 h, regarding germination percentage, were INCASoy-24 and INCASoy-27. In radicle length and mass of 100 radicles, the cultivar INCASoy-2 was the best one.

To evaluate under field conditions the best treatments on diverse cultivars and different seasons of crop planting, is recommended.

Acknowledgements

The authors thank the Local Agricultural Innovation Project and the entire team of Local Agricultural Innovation for facilitating the information, means and equipment used in the research.

Conflict of interests

There is no conflict of interests between the authors.

Table 4. Dry mass of 100 radicles (mg) at 120 h after putting the seeds to germinate, subject to 50 °C during three times.

Cultivar	120 h after being put to germinate			
	Control	1 h 50 °C	5 h 50 °C	10 h 50 °C
INCASoy-1	45,6	60,7	105,2	38
INCASoy-2	47,2	62,3	114,2	38,1
INCASoy-24	46,3	60,2	102,3	35,4
INCASoy-27	46,7	62,4	110,2	37,4
INCASoy-35	45,2	60,2	106	35,1
INCASoy-36	44,5	59,4	100,4	37,2
CIGB L-1	47,1	59,4	108,4	37
Carolina RP-5	46,6	61,3	108,6	36
CEB-2	46,0	60,4	107,8	35,4
Mean value	46,1[c]	60,7[b]	107,0[a]	36,6[d]
VC %	5,63			
SE ±	0,4452			

Authors' contribution

- Alejandro Mederos-Ramírez. Conceptualization, data curation, formal analysis, acquisition of funds, research, methodology, validation, administration of the project resources, manuscript writing and revision.
- Rodobaldo Ortiz-Pérez. Conceptualization, data curation, formal analysis, acquisition of funds, research, methodology, administration of the project resources, supervision, validation, visualization, manuscript writing and revision.

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