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Original article

Germination of bean seeds (*Phaseolus vulgaris* L.) at different temperatures

Germinación de semillas de frijol (*Phaseolus vulgaris* L.) a diferentes temperaturas

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ABSTRACT: The research was developed in the Base Scientific Science Unit, Los Palacios, Pinar del Río, Cuba, belonging to the National Institute of Agricultural Sciences. The objective was to evaluate the influence of temperatures on the germination of four bean cultivars. Seeds of *Phaseolus vulgaris* L. (beans) were analyzed from the cultivars, Cubana 23, Chévere, Buena Ventura, La Cuba 154. The germination temperatures considered in each trial were 20, 30 and 40 ºC. The experimental design used was completely randomized in all cases, each trial was evaluated individually and was repeated twice, it worked with the means of these repetitions. Germinated seeds were counted from their establishment until stabilization, with the obtained data: germination percentage, germinated seeds per day and the Maguire Index were determined. The results were subjected to an analysis of variance (ANOVA) with a confidence level of 99 %. The results of this work indicate that there is sensitivity of different bean cultivars to a variation in temperature in the germination process; therefore, the effect of temperature is closely related to the genetic material that is being worked on. Temperatures higher than 30 ºC decrease the germination speed in cultivars under study.

Key words: germination rate, legume, vigor.

RESUMEN: La investigación se desarrolló en la Unidad Científico Tecnológica de Base, Los Palacios, Pinar del Río, Cuba, perteneciente al Instituto Nacional de Ciencias Agrícolas. El objetivo fue evaluar la influencia de diferentes temperaturas en la germinación de cuatro cultivares de frijol. Se analizaron semillas de *Phaseolus vulgaris* L. (frijol) de los cultivares, Cubana 23, Chévere, Buena Ventura, La Cuba 154. Las temperaturas de germinación consideradas en cada ensayo fueron 20, 30 y 40 ºC. El diseño experimental utilizado en todos los casos fue completamente aleatorizado, cada ensayo fue evaluado individualmente y fue repetido dos veces, se trabajó con los valores medios de dichas repeticiones. Se contaron las semillas germinadas desde su establecimiento hasta la estabilización, con los datos obtenidos se determinó: porcentaje de germinación; número de semillas germinadas por día y el Índice Maguire. Los resultados fueron sometidos a un análisis de varianza (ANOVA) con un nivel de confianza del 99 %. Los resultados de este trabajo indican que existen sensibilidad de diferentes cultivares de frijol ante una variación en la temperatura, durante el proceso de germinación; por tanto, el efecto de la temperatura está estrechamente relacionado con el material genético con que se está trabajando. Temperaturas superiores a 30 ºC disminuyen la velocidad de germinación en los cultivares en estudio.

Palabras clave: índice de germinación, leguminosas, vigor.

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INTRODUCTION

The *Phaseolus vulgaris* L bean is one of the legumes that has a preferential place, due to its nutritional composition as it is a rich source of proteins and minerals (1,2). It is an annual legume, intensively cultivated from the tropics to the temperate zones (3,4). It occupies more than 80 % of the area sown annually in the world with approximately 15 million hectares (5).

In Cuba, this legume constitutes a fundamental dish in the diet of the settlers, where together with rice (*Oryza sativa* L.) It forms part of the basic diet. However, the country has been importing more than 60 thousand tons of grain per year in order to satisfy market demand (6,7). That is why strategies are followed to increase yields and reduce imports, for which large areas have been allocated both in the state sector and in farmers' farms. The implementation of actions such as promoting the genetic breeding of cultivars, seeds´ production and the achievement of a correct phytotechnics with emphasis on achieving adequate germination and guaranteeing the density of plants established by the cultivation instructions is also strengthened. However, the response of bean cultivars to different environmental conditions due to the great variability of the climate is currently an element of vital importance to achieve high productivity in the face of climate change effects (8). Therefore, to achieve stable yields over time or to increase them, it is necessary to analyze which are the main factors that contribute to determining the final yield, to know their influence and to carry out an adequate management of them. This is because beans require relatively low temperatures for their normal development and when these are high, the germination, morphology and growth of plants are affected, ultimately limiting their productivity. For this reason in Cuba, its cultivation is generally limited to a short period. Furthermore, despite the existence of approximately 21 registered cultivars in the country, the information on their response to climate change effects is insufficient (9). It is evident that temperature is one of the most important environmental factors during bean plant development and the one that is affecting it the most because of climate change incidence. That is why research should be conducted in order to identify bean cultivars most adapted to the existing climate variability, especially to the increase in temperature.

From the phases and cultivation stages, the germination process is considered one of the most affected by temperature due to its effect on the activity of enzymes that regulate the speed of biochemical reactions that occur in the seed after rehydration (10). Currently there is little information on temperature effect on bean cultivar germination obtained in the country and a precise understanding of germination dynamics at different temperatures is required to ensure a number of seedlings in productive practice. Furthermore, this experiment is important to promote programs to improve cultivars based on adaptation to the climate in agricultural sector. Due to the aforementioned, this work was carried out with the aim of evaluating the influence of temperatures on the germination of four bean cultivars.

MATERIALS AND METHODS

The experiments were carried out at the Los Palacios Base Scientific and Technological Unit (UCTB-LP), belonging to the National Institute of Agricultural Sciences; located in the southern plain of Pinar del Río Province, at 22° 44' north latitude and 83° 45' west latitude, at 60 m a.s.l, with an approximate slope of 1 %, according to the Atlas of Cuba (3). *Phaseolus vulgaris* L. seeds (bean) from four cultivars were analyzed, Cubana 23 (black color), Chévere (white color), Buena Ventura (red color), La Cuba 154 (beige color) (Table 1) (9). These were supplied by the seed group of the National Institute of Agricultural Sciences. For their selection, the availability of seeds, the area sown at least in the western provinces, grain color and growth habit were taken into account.

Once the crop was harvested, and with the purpose of lowering its humidity to 10 % to avoid deterioration, the pods were dried in a forced air oven at 40 °C for 48 hours, and later they were dehulled. The seed obtained was placed on sieves with circular holes, and the one that was retained between the 0.7 to 1.6 cm long sieves that was used in the experimentation. This caliber corresponded to the middle third of the different sizes of seeds obtained in each cultivar and was the most representative of each one. Before being subjected to germination tests.

In the experiment, the germination temperatures considered in each test were 20, 30 and 40 ºC. The experimental unit corresponded to glass Petri dishes 140 mm in diameter and 20 mm high, with 2 layers of filter paper moistened with distilled water on the bottom of the plates and 30 seeds inside. Four repetitions per treatment were established. Once the seeds were placed in the plates, they were placed in a germination chamber model RTOP-310D regulated at the respective temperature (20, 30, 40 ^{o}C).

Table 1. Table of cultivars´ description.

Cultivars	CG	Potential yield (t ha^{-1})	GH	Days after sowing			Mass 100 seeds (grams)
				DF	DPM	DHM	
Cubana 23	Black	1.2	II	43	75	85	19
Buena Ventura	Red	2.9	II	33	68	79	19
Chévere	White	3.1	III	39	71	81	18
La Cuba 154	White	3.0	III	35	70	85	18

CG: Color of the grain; GH: growth habit; DF: Days to flowering; DPM: Days to physiological maturity; DHM: Days to harvest maturity

The experimental design used in all cases was completely randomized, each trial (Temperatures 20, 30, 40 ºC) was evaluated individually and was repeated twice, working with the means of said repetitions.

Germinated seeds were counted from their establishment to stabilization, with the data obtained it was determined:

- Germination percentage (GP): Germinated seed was considered to be oone with a radicle of length greater than or equal to 2 mm (11).

$$PG = \frac{Número\ de\ semillas\ germinadas}{Número\ de\ semillas\ sembradas} \times 100 \qquad (1)$$

- Germinated seeds per day (GSD): Germinated seeds were counted daily and the appearance of a radicle greater than or equal to 2 mm was used as a criterion.
- Maguire Index (MI): Represents the germination speed calculated through a weighted accumulated germination time. Where G is the percentage of seedlings that germinated during the time interval t (12).

$$IM = \sum_{t=1}^{n} \frac{G_1}{t_1} \qquad (2)$$

The assumptions of normality and variance homogeneity were checked for the data of each evaluated variable (Bartlett's Test and Kolmogorov-Smirnov, respectively). The results (GP, MI) were subjected to an analysis of variance (ANOVA) with a confidence level of 99 %. Comparison of means was made using Duncan's Multiple Ranges Test (P 0.01) (13). For this, the STATGAPHICS Centurión Program on Windows, version XV (14) was used. With the values of germinated seeds per day, graphs of the germination dynamics of each cultivar were made at the different temperatures studied.

RESULTS AND DISCUSSION

The final germination percentages appear in Table 2, all cultivars presented significant differences in the range of 20 to 40 ºC of temperatures. The highest values were observed at 20 ºC, while the lowest were observed at 40 ºC. However, cultivars Cubana 23 and Buena Ventura were more affected by subjecting their seeds to temperatures of 30 ºC, since only 34 and 47 % of the seeds germinated at this temperature, while La Cuba 154 and Chévere presented values of 82 and 73 % respectively.

The results of La Cuba 154 and Chévere at 30 ºC should be taken into account for the planning of the sowing of the crop in Cuba at late dates (after March), and also as progenitors in the genetic improvement programs of the bean crop for resistance to abiotic stresses especially for high temperatures.

The influence of temperature on the seed germination percentage of agricultural interest crops has been reported by several authors, who highlight that the seed emergence percentage guarantees 50 % of the production success and point out that germination only occurs appropriately within a certain temperature range (15,16). In studies carried out in the cultivation of peanuts (*Arachis hypogaea* L), it was shown in all the genotypes studied that germination increased with the rise in temperature above 14 ºC. For the range between 16 and 32 ºC, germination percentages were obtained that meet the standard of commercialization of common seed for peanuts (> 80 %); being in all genotypes equal to or greater than 90 % (17).

It should be noted that in the results of this study, there is a differential and particular response of cultivars to germination temperature and there is variation in the final germination percentages.

Regarding the germination dynamics, there are differences between necessary times for the germination process at the different temperatures to which the seeds of the cultivars under study were exposed (Figure 1). Cubana 23 cultivar shows an advance in the germination beginning of the seeds at 30 ºC in approximately 24 hours, with respect to the temperatures of 20 and 40 ºC that began their germination at 50 hours. However, the behavior of the rest of cultivars at different temperatures was different from that of Cubana 23, since they started the process at approximately 50 hours.

At a temperature of 20 ºC, despite not affecting the germination percentage for cultivars (Cubana 23, Buena Ventura, La Cuba 154, Chévere) (Table 1); It is observed in Figure 1 that the germination dynamics is modified. It is possible to appreciate a delay in the germination process and a long period of approximately 150 and 200 hours necessary for the germination process of seeds to be completed. A similar behavior is observed at 40 ºC, although for this temperature only a prolonged period of approximately 100 to 150 hours is observed, necessary for the germination process of the seeds to complete.

Table 2. Bean seed germination percentages at different temperatures.

	Cultivars			
Temperature (ºC)	**Cubana 23**	**Buena Ventura**	**La Cuba 154**	**Chévere**
20	98.4 a	96.7 a	98.4 a	94.7 a
30	34.2 b	47.5 b	82.5 b	73.3 b
40	12.5 c	19.2 c	12.5 c	15.0 c
SEx	1.7***	1.8***	1.7***	1.2***

Different letters in the same column indicate significant differences (Tukey's test, p≤0.05)

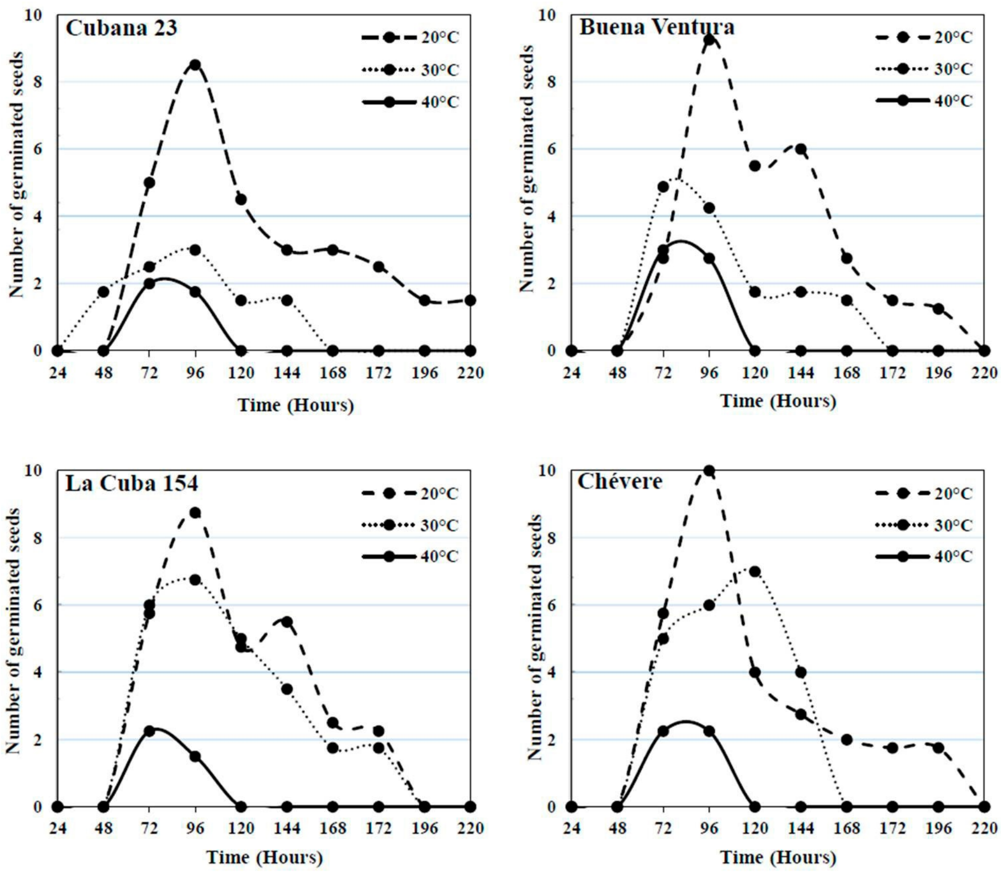

Figure 1. Bean seed germination dynamics of four cultivars at different temperatures.

In this case, this may be related to the low percentage of seed germination at this temperature (Table 2).

The behavior of Cubana 23 cultivar, which advanced the start of seed germination in 24 hours at a temperature of 30 ºC, could be taken into account for subsequent studies in search of cultivars at high temperatures and for the selection of parents in the breeding program Bean genetics looking for abiotic stress tolerance.

In studies with corn seeds (*Zea mays* L.), it was reported that when the temperature increased to 45 ºC, the mean germination time increased (18). However, in tomato seeds (*Lycopersicon esculentum* Mill), despite not affecting germination between 15 and 25 ºC for the cultivars studied, it was observed that the germination dynamics was modified by temperatures and the moment of obtaining the maximum germination speed as they increased. It stands out that at 35 ºC; in the cultivars where the seeds were able to germinate, the germination time was prolonged to approximately 144 hours (19).

The Maguire Index (Figure 2), which is a variable that weights accumulated germination over time, was able to differentiate temperature effect with the germination process of different cultivars. It can be seen in the figure that there are significant differences between the different temperatures for each cultivar.

It was also observed that the highest values of this index were reached with temperatures of 20 ºC for all cultivars under study. However, at 30 and 40 ºC they had a lower index. Despite this, it can be specified that at 30 ºC Cubana 23 and Buena Ventura cultivars are observed with a better performance than other cultivars in studies. In the climatic conditions of Cuba, the problem that exists in terms of germination reduction at temperatures above 30 ºC is of great interest, there are studies where seed germination indices are used for the early selection of genotypes tolerant to certain stress (16,20).

Aspects pointed out by some authors, report that poor seed germination at high temperatures is related to protein synthesis in the seed embryo, which influences their germination speed. In this regard, it is reported that several processes occur in the seed that depend solely on reserves, the gibberellic acid of the embryo acts on the aleurone layer where the amylase enzyme is activated, which initiates the degradation of reserve substances contained in the endosperm and cotyledon. From the digestion of reserve tissues, various compounds are reused in multiple synthesis processes. Complex molecules such as celluloses, hemicelluloses, starches, amylopectins, lipids, lignins, proteins, nucleic acids, vitamins and hormones, are degraded to simple molecules by specific enzymes (11,21). Other authors highlight the pericarp role in the thermo inhibition of the seeds, they point out that this is due to the presence of inhibitors in the tissue or also because in the high temperature presence the oxygen requirements are relatively high, it does not allow the pericarp to maintain these demands (22).

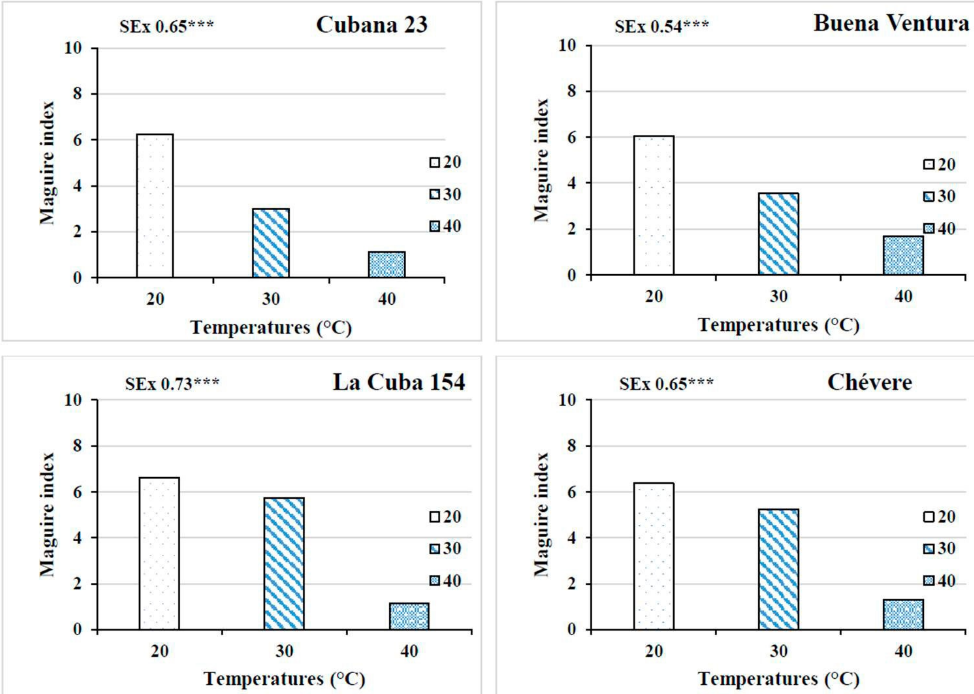

Figure 2. Maguire index of bean seeds from four cultivars at different temperatures.

For most cases, the germination speed increases with increasing temperature. Although very high temperatures also tend to decrease it, in this range it is possible to find the optimum germination temperature. With temperatures above 32 ºC, the germination speed index of seeds can decrease. However, regardless of the genotype, the increase in temperature up to 38 ºC significantly reduces the germination speed (22,23). High temperature negative effect on the germination speed was also exposed by some authors (24), who pointed out that once the optimum temperature level is reached, where the germination speed is higher, a decrease occurs as the temperatures rise approach their maximum limit where irreversible damage occurs to seeds.

CONCLUSIONS

- The results of this work indicate that there are sensitivity of different bean cultivars to a variation in temperature in the germination process; therefore, the effect of temperature is closely related to the genetic material with which is used.
- Temperatures above 30 ºC decrease the speed of germination in the cultivars under study.

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