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High temperatures effect on morpho-physiological indicators in common bean (*Phaseolus vulgaris* L.) plants during the germination and growth in non-optimum season

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Abstract: The knowledge about high temperature effect on plant crop is important for adaptation strategies to Climate Change. For this reason the aim of the paper was to analyze its abiotic effect on common bean cultivation in a Red Ferralitic soil. A sowing of twelve varieties was performed in non-optimum season (high heat environment) during three years: 2013, 2014 and 2015 through an alpha-Latin design using three replicates per accession. The number of germinated plants was measured within the first 11 days after sowing to obtain the survival percentage. In the same way absolute (AGR) and relative (RGR) growth rate between 21 and 30 days and the plant height from 20 to 70 days with measurement ranges of 10 and 15 days were also evaluated and plotted by a logistic model. The values of daily mean temperature (DMT) were registered to calculate the accumulative temperature during the germination and growth phases. For the statistical evaluation, AGR and RGR were analyzed by one-way analysis of variance and a Tukey comparison where significance was defined with a probability level of $P<0.05$. The results showed that plant survival percentage was significantly affected by increasing temperature following a sigmoid model, where differences among varieties were observed, while a prediction of survival behavior was also carried out for extreme values of temperature. The model corroborated that DMT above 28 °C decreases the survival percentage until values less than 30%. On the other hand the most sensible varieties in the germination stage showed a higher relative growth rate which contributes for understanding the physiological effect of thermal stress in common bean plants.

Keywords: agriculture; climatic change; model; temperature.

INTRODUCTION

Abiotic factors like humidity, temperature and soil salinity impact on the agricultural production. The knowledge about the incidence of different meteorological parameters on plant yield is developed considering a future scenario within the Climate Change[1].

In relation to temperature, there are predictions about the increase from 3 to 4 °C, and it is added to the historic increment of 0.6 °C caused by the continuous emission of greenhouse effect gases[2]. The negative repercussion of these temperature increases on agricultural production has been anticipated according to prediction models, where thermal stress caused by high environmental temperature above the historical average has affected the yields [3-5.] In a same way a relevant relationship between metabolic pathway process and the grain filling stage has been reported in several species [6] as well as molecular responses involved in acclimation to high heat blow[7] .

Temperature values, above specific optimum values for each accession, negatively impact on the growth and development process which rebound in a late date to reach to the different phenological stages[8]. These effects have been quantified by the calculus of "thermal factor (F_T)" or "accumulative temperature" for the different phases, through the daily addition of the temperature average values. Previous papers have found gens for controlling the plant response within thermal stress, fundamentally related with the physiological process during the arrival date of specific phases like the flowering[4]. In some cases, the flowering and maturation arrival could be related with previous phases like growth and survival rate post-germination. The regulatory mechanisms of these responses could also be linked to several processes where the participation of auxin is suggested[9]. The aim of this paper is the quantification of the stress effects caused by high temperatures on *P. vulgaris*, through the sowing in non-optimum season during germination and growth stages and its replication during three years.

MATERIAL AND METHODS

Theoretical aspects

Thermal factor (F_T) or accumulative temperature: The thermal factor (F_T) should firstly be defined. If the daily mean temperature T_j is measured during N days, then the addition of all these values $T_1+T_2+....+T_N$ allows the obtaining of total average value multiplied by N. Nevertheless when we observe the harmful effects of high temperature within a certain time range (like a specific phase in a life cycle), is important to indicate that only the values higher than T_U called "threshold value" can impact on plant response, while values for $T_j<T_U$ do not negatively rebound in any way. T_U value is composed by two parameters: the "base

temperature" (T_{Base}) and the non-invasive increment ΔT_Q which represents the values that added to the T_{Base} obtains the threshold value T_U without affect the physiological process. For example in *P. vulgaris* the value 15 °C is chosen as base temperature according to the criterion endorsed by experiments[10]. On the other hand the optimum value depends on the climatic region and the accession. For Cuban conditions and usually managed varieties it is 25°C, which corresponds to the daily mean temperature in the optimum season for common bean sowing (October). Therefore, ΔT_Q=10 °C is estimated and following this reasoning the values of 27°C is related to the daily increment of 2°C above threshold value. This increment will be called 'invasive' ($\Delta T'$) because it affects the plant development. For this reason, the estimation of parameters that contains these increments in a range of *N* days is convenient, but at the same time it should work on the base temperature instead threshold value, because the threshold temperature in not the same value for all varieties and regions. For *N* days, F_T will be defined as the daily difference between average temperature and base value[11]:

$$F_T = \sum_{j=1}^{N} \left(T_j - T_{Base} \right) \qquad (1)$$

Where the following relationship was already defined:

$$T_j = T_{Base} + \Delta T_Q + \Delta T'_j \qquad (2)$$

From this concept the effect of the climatic change can be managed regarding the temperature for evaluating the impacts caused with respect to a standard level. F_T represents the area under the curve from a geometric point of view, when is plotted with respect to the numbers of days. Therefore it may be represented in °C·day, although in a lot of papers it is expressed only in °C units[11-13].

Temperature and germination: In the analyzing of germination capacity "*g*" was defined as the magnitude to represent it in decimal values (between 0 and 1) and $<\Delta T'>$ the invasive temperature average in *N* days. The derivate of *g* with respect to $<\Delta T'>$ can be expressed:

$$\frac{dg}{d < \Delta T' >} = N \cdot f(g) < 0 \qquad (3)$$

Where *f(g)* is a continuous and derivatively function for all de domain from 0 until 1. This function is negative because the $<\Delta T'>$ increasing effects provokes a decreasing "*g*" which also increases its value regarding *N*. That is to say a high number of days combined with a high temperature value provoke intensification the g decreasing. For low values of *g* a polynomial 2-degree may be considered for the function *f(g)*:

$$f(g) = -g(1-g)K \qquad (4)$$

Here *K* is a constant. After the substitution of (4) in (3):

$$\frac{dg}{d < \Delta T' >} = -N \cdot g(1-g)K$$

$$\frac{dg}{d < \Delta T' >} = -N \cdot g^2 \left(\frac{1}{g} - 1 \right) K \qquad (5)$$

Finally is very easy to find the solution for (5) in the form:

$$g = \frac{1}{1 + AExp\left[K \cdot N < \Delta T >\right]} \qquad (6)$$

“A” is integration constant. From (1) and (2) we may write:

$$F_T = \sum_{j=1}^{N}\left(T_j - T_{Base}\right) = \sum_{j=1}^{N}\left(\Delta T_Q + \Delta T'_j\right) = N \cdot \Delta T_Q + \sum_{j=1}^{N}\left(\Delta T'_j\right)$$

$$F_T = N \cdot \Delta T_Q + N \cdot < \Delta T'_j > \qquad (7)$$

Substituting (7) in (6):

$$g = \frac{1}{1 + AExp\left[K \cdot \left(F_T - N \cdot \Delta T_Q\right)\right]} \qquad (8)$$

The reduced logistic equation is obtained:

$$g = \frac{1}{1 + bExp\left[K \cdot F_T\right]} \qquad (9)$$

Where:

$$b = Exp\left[\ln(A) - K \cdot N \cdot \Delta T_Q\right] \quad (10)$$

The auto-catalytic logistic equation (9) informs that the behavior of g with respect to F_T is sigmoid. For daily mean temperature the F_T value sufficiently increases to provoke a decreasing on germination until 0. In the experimental plotting $F_T = N\Delta T_Q$ is keeping in mind to consider that g maintain its values near of 1 (germination 100%). On the other hand, (9) allows quantifying the necessary value in the F_T for decreasing the germination until 50%.

$$F_{T50} = -\frac{\ln(b)}{K} \qquad (11)$$

EXPERIMENTAL DEVELOPMENT

Germination: Twelve varieties of common bean were used (*Phaseolus vulgaris* L.; cv: P30L50; P646; P871; P667; P882; P662; P1185; Caujerí 2170; Rayado 2258; CC25-9R; CC25-9N; Lewa), seven provided from INIFAT genebank and the rest is commercial material. The sowing was carried out by an Alpha-Latin design (in three replicates) and the date was chosen in non-optimum season so the highest temperature in August coincided with the reproductive phase. For this reason the life cycle was planned in three rainy and high blow heat periods (July-September). The sowing dates were June 27th for 2013 and July 2nd in 2014 and 2015.The initial seed germination was 99-100% with one seed per 10 cm at furrow wide of 90 cm according the technical instructive[14, 15].

The number of germinate plants was evaluated for calculation of the survival percentage at 11 days after sowing. The temperature values were collected in the Meteorological Station located in Santiago de Las Vegas (300 m from the experimental site). In this way survival percentage was related with F_T using the daily mean temperature during the first 12 days.

The relationship between S and F_T was fitted by the following equation:

$$S = \frac{100\%}{1 + b \cdot Exp[K \cdot F_T]} \qquad (12)$$

This was obtained from (9) while F_T was calculated during the germination phase for each year. The base temperature was selected in 15 °C, considering the optimum mean temperature for the growth[10]. According to the varieties employed and the daily mean temperature in optimum season (25 °C) the parameter T_Q was estimated in 10°C.

Growth phase: For evaluating the growth rate five plants per parcel were collected (15 in total per accession) at 21 and 30 days after sowing and the material was daily placed in a stove in 80 °C during eight hours for three days to obtain the dry mass value for each one. The absolute (AGR) and relative (RGR) growth rate were calculated between both days by the following equations[16]:

$$AGR = \frac{MS_2 - MS_1}{t_2 - t_1} \quad (13) \qquad RGR = \frac{\ln[MS_2] - \ln[MS_1]}{t_2 - t_1} \quad (14)$$

Where MS_1 and MS_2 are the dry mass per plant evaluated at the respective days after sowing t_1=21 and t_2=30 days. For statistical comparison *AGR* and *RGR* values were subjected in the one factorial variance analysis with respect to accession while the mean values were compared by a Tukey test considering $P<0.05$ as significant, using a version of SSPS software for Windows. On the other hand a correlation analysis was carried out between the parameters *RGR* and *K* using the values obtained by fitting of equation (12). The plant height during the life cycle from 20 to 70 days with measurement ranges of 10-15 days was evaluated while the data was processed through CURVE-Expert program (version 1.3), for obtaining the mathematical fitted parameters by a logistical model according to the expression:

$$h = \frac{h_{\max}}{1 + \delta \cdot Exp[-\sigma \cdot t]} \qquad (15)$$

Where h_{max} is the maximum height for the plan while δ and σ are constants.

RESULTS

Germination phase: The two-factorial variance analysis showed that accession, year and the twice interaction impacted significantly on the survival percentage. The differences observed in all the varieties with respect to the year are caused by the temperature values which are different in each season. **Figure 1** shows how is the behavior of the mean temperature (measured for each 10 days) in 2014 and 2015 with respect to 2013. These values show the tendency for increasing in F_T regarding the years.

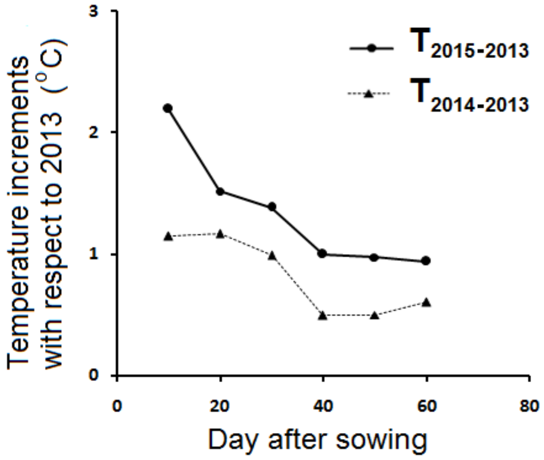

Figure 1: Temperature increment values per day (averaged for ten days) for the years 2014 and 2015 with respect to 2013.

With these values a tendency for increasing in F_T was observed (calculated for the first 12 days) 129.4 °C, 141.1 °C and 153.2 °C for 2013, 2014 and 2015 years, respectively. Figure 2 reflects the high good fitting (regression parameters in Table 1) for the points, according to the proposed model.

Table 1: Coefficient values for the survival percentage (fitted by Eq. (12)).

Accession	b (10^{-9})	K ($°C^{-1}$)	F_{T50} (°C)	r^2
Caujerí2170	1.30	0.1420	144.1	0.966
P882	4.00	0.1293	149.5	0.996
P30L50	6.92	0.1292	145.4	0.907
P662	1.15	0.1396	147.5	0.988
P646	1.03	0.1395	148.3	0.985
CC25-9R	0.80	0.1407	148.9	0.932
Rayado2258	8.10	0.1247	149.4	0.807
Lewa	1.29	0.1417	144.5	0.997
P871	0.92	0.1395	149.1	0.968
P1185	0.86	0.1370	152.4	0.901
P667	1.27	0.1402	146.1	0.971
CC25-9N	1.31	0.1378	148.4	0.933

The r^2 values (above 0.9) represent a high approximation between theoretical function and experiment for all the varieties except "Rayado2258" (r^2=0.80). For varieties P1185, P882 and Rayado2258, the higher values of F_{T50} were obtained 152.4, 149.5 y 149.4 °C, respectively, which indicates varieties that show a significant tolerance to survival in the first days on life cycle with increasing temperature above the optimum value. These values represent that with a 12 day’s summary of mean temperature of 27.7 °C (12.7 °C above base temperature and 2.7 above the optimum value) the varieties like P1185 can produce the 50% of the sowed plants with an initial germination percentage of 100%. P1185 could be tolerant in an environment with 2.7 °C above the optimum while Caujerí2170 loses the germination half with 2.0 °C. The sigmoid curve allows predicting how the survival percentage variation is related with environmental temperature increments.

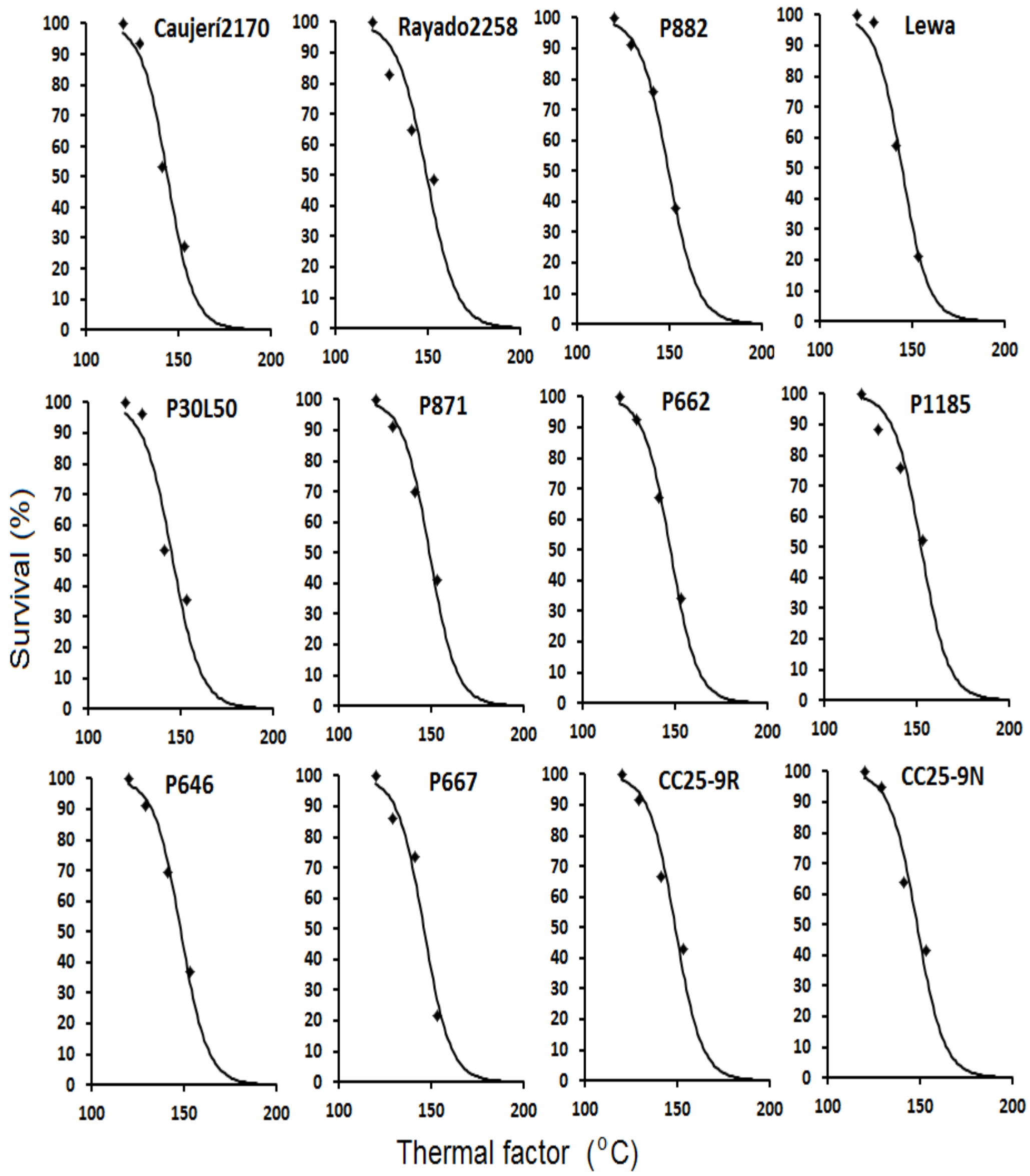

Figure 2: Survival percentage value decreases progressively to zero with increasing thermal factor. The initial F_T value (survival of 100%) is corresponded with the threshold value at 25 °C. The dots represent the experimental data for each season while the curves show the mathematical fitting by the equation (2).

The Figure 3 shows each value of the survival with respect to a value of daily mean temperature, supposing that it is constant during the first 12 days. In the figure the varieties Caujerí 2170 and P1185 are represented (highest and lowest values of F_{T50} respectively). In the plant related to both varieties to constant temperature of 28 °C was appreciated a decreasing significantly below 30%, according with this simulation.

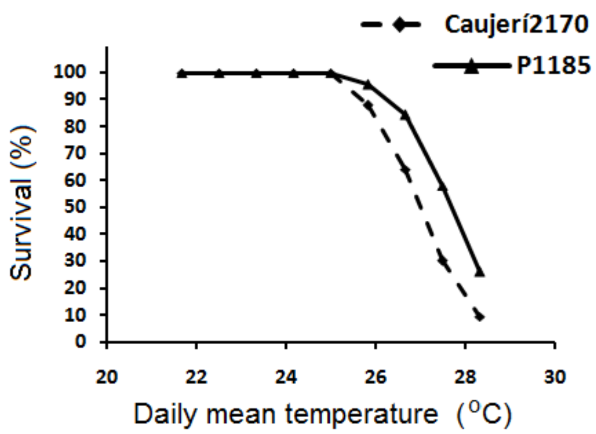

Figure 3: Theoretical curves of the survival percentage with respect to the daily mean temperature for the varieties Caujerí2170 and P1185.

Growth phase: The Table 2 reflects the behavior of the absolute (*AGR*) and relative (*RGR*) growth rate for all the varieties. The marked difference by variance analysis performed shows several varieties with tolerance to growth under high temperatures. Varieties like P1185 and P871 produced a highest dry mass values between 21 and 30 days after sowing.

Table 2: Values of growth rate: absolute (*AGR*) and relative (*RGR*), evaluated between 21 and 30 days after sowing. The values are showed with the typical error, while different letters represent significant differences between varieties according the Tukey test (P<0.05).

Accession	AGR(g·day^{-1})		RGR (day^{-1})	
Caujerí2170	0.144±0.013	ab	0.128±0.007	b
Rayado2258	0.090±0.007	b	0.090±0.005	c
P882	0.154±0.018	a	0.119±0.012	b
Lewa	0.134±0.004	ab	0.131±0.006	ab
P30L50	0.123±0.019	ab	0.123±0.011	b
P871	0.156±0.019	a	0.140±0.012	a
P662	0.134±0.019	ab	0.124±0.013	b
P1185	0.173±0.018	a	0.134±0.007	a
P646	0.108±0.007	b	0.119±0.005	b
P667	0.165±0.004	ab	0.130±0.004	b
CC25-9R	0.110±0.011	b	0.122±0.007	b
CC25-9N	0.106±0.009	b	0.134±0.009	a

***N*=15 (three replicates and five plants were taken in each one)**

K coefficient represents the dynamic of germination loss with increasing temperature. Figure 4 shows the relationship between *K* and *RGR* where a significant correlation was obtained, according to the analysis performed.

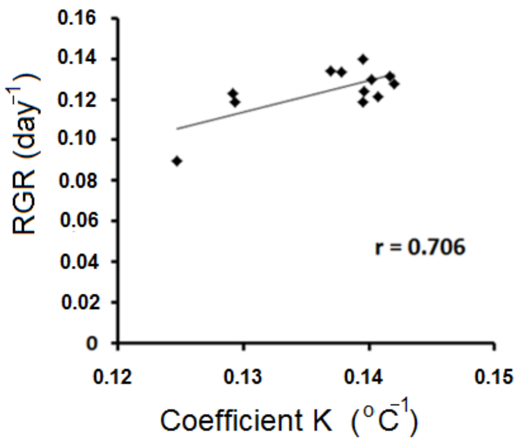

Figure 4: Significant relationship between the relative growth rate (*RGR*) and the germination thermal susceptibility derived from the linear correlation analysis.

This proportional directly relationship demonstrates that under thermal stress situation there is a certain inverse link between the seedling germination response during the first 12 days (when the nutrients are coming from the cotyledons) and the plant capacity to growth through incorporating of dry mass by photosynthesis and mineral nutrition in later stages. This results also reflects that varieties with a high K values (higher susceptibility to decreasing the survival percentage caused by temperature effect) showed the high *RGR* values. This outcome may be explained because the plant subjected to this kind of stress can use this stimulus for the potentiating the physiological process during the growth.

The plant height measured during the growth phase at seasons 2013 and 2014 are shown in Figure 5. In all cases a sigmoid behavior is obtained according to the equation (15) (solid and dashed lines) where the maximum values is reached lower than the value in optimum season for each accession (dot-streak lines). The mathematical fitting by (15) shows that both seasons, the variety Rayado2258 reached the time t_{50} (moment when the plant arrives to the half maximum height during the growth) earlier than the rest of the varieties.

The thermal factor in 2014 contains the highest values because the daily mean temperature in this year was higher than 2013 during the first 45 days (Fig. 1). For this reason t_{50} values in 2014, (which represent replies with respect to 2013), show the highest values including Rayado2258 with t_{50}=27.7 and 33.3 days in 2013 and 2014 respectively.

Table 3 represents F_T values calculated until t_{50}, where variations between varieties are observed and it shows how the thermal effect decreasing the growth speed in plants at the same time when the final height is below regarding the optimum season value. Nevertheless the correlation between h_{max} / h_{op} and F_T was not significant. In the same way neither was obtained a significant relationship between final height h_{max} and the growth rate.

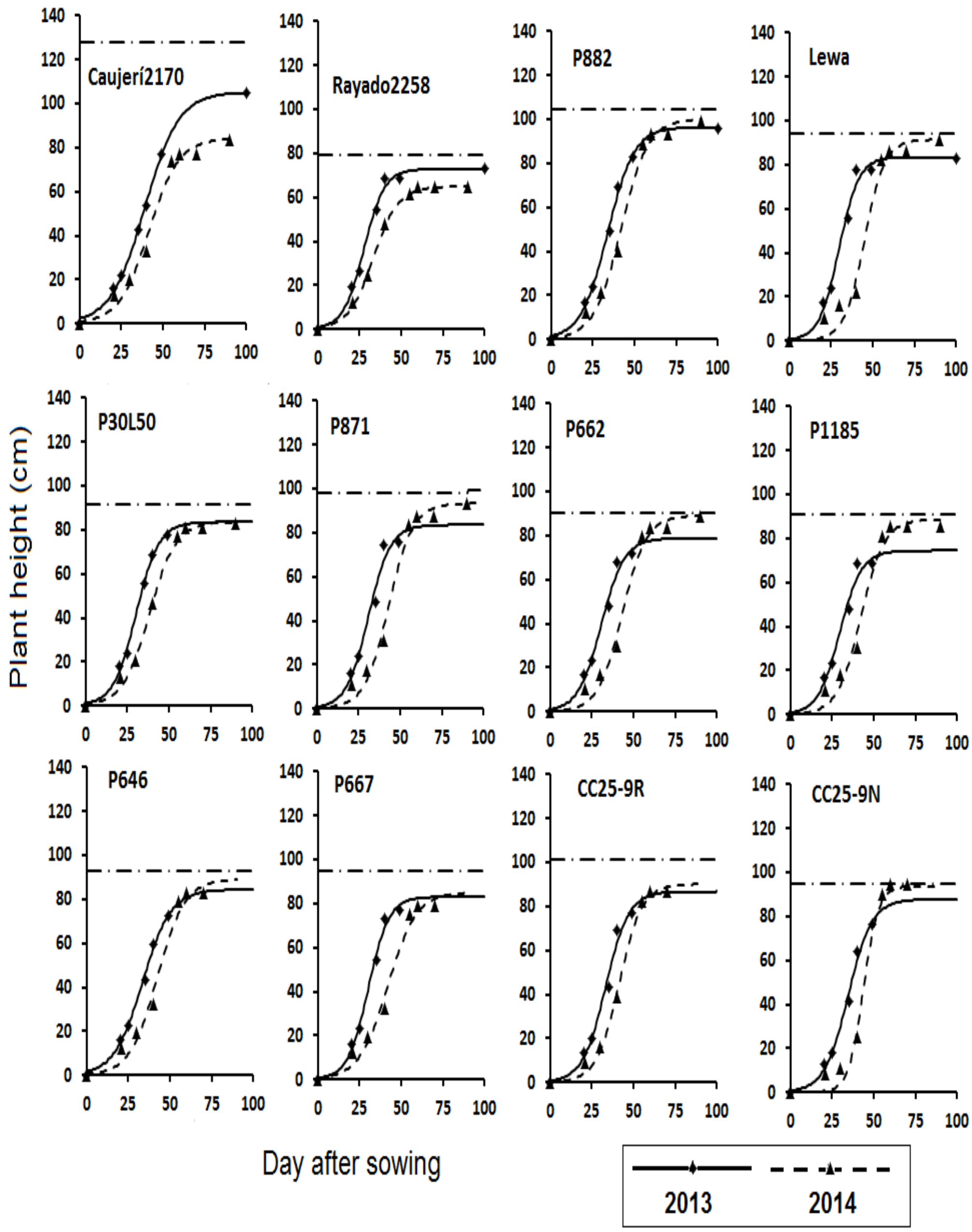

Figure 5: Values of plant height during the season 2013 and 2014.The dots shows the experimental values while the lines are the growth curves fitted by (15)

Table 3: Model coefficients values (equation 15) obtained by fitting (seasons 2013-2014).

Accession	2013 σ (day^{-1})	2013 t_{50} (day)	2013 $F_T(t_{50})$ (°C)	2013 h_{max} (cm)	2013 h_{op} (cm)	2014 σ (day^{-1})	2014 t_{50} (day)	2014 $F_T(t_{50})$ (°C)	2014 h_{MAX} (cm)	2014 h_{op} (cm)
Caujerí2170	0.095	40.0	456.9	109.1	127.7	0.112	41.6	474.6	84.2	127.7
Rayado2258	0.163	27.7	312.0	72.3	79.11	0.134	33.3	377.6	64.9	79.11
P882	0.127	34.0	386.3	96.4	104.4	0.123	41.9	478.2	100.3	104.4
Lewa	0.170	29.9	337.6	83.1	94.11	0.165	45.0	515.2	91.4	94.11
P30L50	0.147	30.4	343.4	82.8	91.67	0.127	38.2	435.2	83.5	91.67
P871	0.157	31.0	350.7	82.5	98.1	0.139	43.1	492.4	93.3	98.1
P662	0.146	30.8	348.9	78.0	90	0.139	43.2	494.3	89.1	90
P1185	0.154	29.8	336.6	74.3	91	0.143	42.3	483.5	88.6	91
P646	0.114	34.1	387.1	85.8	93	0.122	42.4	484.5	89.1	93
P667	0.159	30.5	344.6	83.1	95.17	0.117	41.9	478.5	85.2	95.17
CC25-9R	0.147	33.2	376.3	85.5	101.5	0.148	41.4	472.9	90.1	101.5
CC25-9N	0.145	34.5	392.3	86.8	95	0.225	43.8	500.6	93.8	95

DISCUSSION

One of the factors to keep in mind in the result analysis contains the aspect related with the sowing in warm-humidity season for *P. vulgaris*. In this cultivation period exists other predominant factors which affect the plant response like the abundant rainfall during the months June-September[18,19] . Common bean is cultivated in low rainfall season (November-February), with sowing in October-November preferably, even some genotypic materials tested like Rayado2258 has showed effects on other kind of stress like drought[20] . The germination and growth process under field conditions have corroborated the high temperature effect on the germination capacity of the seed embryo tissue[20].

The sensibility to biochemical reaction regarding the temperature has a considerable influence in these processes likewise extreme temperatures can cause damage in cell and tissues and favors the enzymatic protein dissociation in cellular membranes [21]. In a same way the high temperature reduces the germination percentage (thermal-inhibition) on *Arabidopsis thaliana* plants. These effects can be explained by the incidence of this stress on the growth regulator controllers like Abscise acid (ABA) and the Gibberellins (GAs)[22, 23].

This plant species possess a high tolerance to temperature changes so several molecular mechanisms have been identified like factors related to the thermo-tolerance [7]. The idea about existence of responsible genes in provoking effects to high temperatures is sustained[4], which may also be related with the auxin production[9]. Varieties with a high tolerance to high temperatures in germination stages presented a lowest relative growth rate which allows supposing that in stress situation plant experiments a development in certain process in expense to affect others.

The sigmoid model to represent the temperature effects on survival percentage was fitted in an r^2 value above 0.8 in all the cases and it may be used to predicting the extreme temperatures to plant survival. Although in the present research F_T was calculated by the daily mean temperature, maximum temperature values of 30 °C or more, in determined hours of day, should be considered. The mathematical model proposed has the advantage of the calculation of the physiological effect on germination in a range of daily temperature values (first 12 values of mean temperatures) and not considered a single value of them just as the establishing of simulations in the prolonged heat impacts, due to the presence of high temperatures in wide range day even in the covering a whole crop phase.

There is a narrow relationship between leaf temperature and the water regime. When the air temperature increases the water potentials in the common bean plant up region are trending to decrease and cause the delay in the arriving to flowering phase and therefore the yields is affected[24]. High temperatures also impact on the water ascent by the stem because plants subjected in the water or thermal stress suffer the xylem embolism due to the imperfections in the xylem tube and the temperature according to several models[25, 26].

On the other hand the identification of different tolerance responses in common bean is important to adaptation strategies for Climate Change. The use of a high numbers of accessions contributes to food safety in isolated communities. The negative impact of the Climate Change will be different within spatial localization and intensity, and the all communities are not equality prepared to resist these extreme situations. In the same way, varieties with physiological capacity for the tolerance to abiotic stress are needed for the development of seed production plan in the future. These vulnerable communities generally have not technologic infrastructure for adapting their productive system toward a sustainable management to allow the soil and landscape conservation in harmony with the environment and guarantying the local food safety.

CONCLUSIONS

1. The sigmoid curve model was adequate to represent the survival percentage variation with respect to the thermal factor during the germination phase.
2. Although there were slight differences in K values with respect to the varieties, the survival percentage decreases below 30% with the increasing daily mean temperature above 28 °C, according with the mathematical model.
3. The varieties with a high susceptibility in the germination phase showed a high response during the growth.
4. Mathematical model for growth stage showed the behavior of varieties subjected to high temperatures which decreased the maximum height with respect to the value in optimum season as well as a delaying to reach this maximum value was observed.

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