

Parameters of Physical and Physiological Quality in Tomato Seeds Produced under High Temperature Condition during Different Periods of Development

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ABSTRACT

Objective: To evaluate the quality of the seeds of tomato fruits (*Solanum lycopersicum* Mill.) produced in high temperature (HT) during different phases of development.

Design/Methodology/Approach: Seeds of the Moneymaker variety were planted in a ventilated greenhouse (control treatment, CT) with maximum mean temperature (MMT) of 31.5 °C. A second greenhouse with artificial heating (MMT of 36.5 °C) was used for the HT treatments. When anthesis began from the fourth floral cluster, seven treatments were established: T1) fruits growing permanently in the CT; T2) fruits transferred to HT between five and 12 days after anthesis (daa); T3) fruits growing in HT from 12 to 24 daa; T4) 24-36 daa in HT; T5) 36-48 daa in HT; T6) 48-60 daa in HT; T7) from 60 daa to maturity in HT.

Results: The weight of one thousand seeds (SW) had a positive correlation with the length of seed ($R=0.83^*$), indicating that the increase in SW was primarily determined by an increase in length. The vigor of the seed was measured by the germination after accelerated ageing (GAA); thus, germination and vigor are positively correlated with seed respiration during germination (0.62* and 0.81*, respectively).

Study Limitations/Implications: HT impacting on the second phase of seed development could decrease both the physical and physiological quality of tomato seeds.

Findings/Conclusions: The seeds produced by T7 had lower SW (2.99 g). T5 caused lower amount of seeds per fruit (120), germination (79.4%) and GAA (39.5%).

Keywords: Seed quality, growth, global warming.

INTRODUCTION

The use of high quality seeds constitutes a basic element in agricultural production, which is fundamental in the initial phase of a crop: a high quality seed offers greater possibility for success in seedling establishment (Ayala-Garay *et al.*, 2016), in addition to having higher viability during storage (Pichardo-González *et al.*, 2014). The seed quality includes genetic, phytosanitary, physical (size, volume, etc.), and physiological (viability, germinative capacity, vigor) aspects (Bewley *et al.*, 2013). Seeds are complex structures that have three main components: the embryo that will develop into a vegetative seedling; the endosperm that provides nutrients for the development of the embryo during the first stages of the seedling; and the testa that covers the rest of the components to protect them and to control germination (Taylor, 2020). The start of seed development happens immediately after pollination, when the ovule is activated to begin cell division and histodifferentiation that will form the tissues of the embryo and endosperm. Simultaneously, water flows happen that expand the formed cells and carry compounds that allow the synthesis and storage of reserves. Seed maturation is reached with water loss (tissue desiccation) and the development of the processes that allow the seed to survive with low moisture contents (Taiz *et al.*, 2015). The quality factors are affected by the growth conditions of the parent plant during the phase of seed development.

Temperature is a defining factor on the growth and the different phenological phases of development of the cultivated plants (Hatfield and Prueger, 2015).

The increase in global temperature observed during the last century represents a challenge for agricultural production, since its effects have a substantial impact on the crops' yield (Xu *et al.*, 2017). The response to high temperature depends on the phenological stage of cultivation, and in addition, each species responds to a definite range of maximum and minimum temperatures that form the limits of observable growth (Hatfield and Prueger, 2015). Tomato is one of the most frequently cultivated vegetables in the world; it is used as model plant for the study of Solanaceae and species with berry type fruit (Xu *et al.*, 2017). It is considered a species that is sensitive to temperature increase, the optimal values for the growth and development of the crop range between 25 and 30 °C, while temperatures higher than these are detrimental (Taiz *et al.*, 2015). The increase in

maximum diurnal and nocturnal temperatures, as well as the frequency and duration of the exposure to these, affect negatively the reproductive development and the physiology of tomato (Xu *et al.*, 2017).

There are few studies of the effect of high temperatures during the development of the crop on the quality parameters of tomato seeds (Xu *et al.*, 2017; Delgado-Vargas *et al.*, 2018). Thus, the effect of the affecting high temperature during different periods of seed development on physical (size and weight) and physiological (germination, vigor, respiration, and membrane permeability) variables was evaluated.

MATERIALS AND METHODS

The experiment was carried out in spring-summer in Texcoco, Mexico (19° 27' 51" N and 98° 54' 15" W, at 2250 m of altitude). The genotype used was *Solanum lycopersicum* var. MoneyMaker (MM), of temperate origin, is considered a global reference in tomato studies (Biais *et al.*, 2014; Delgado-Vargas *et al.*, 2018).

One hundred seeds were sown in trays with peat moss number 3 (Sunshine, USA) as substrate, during 40 d. The seedlings were transplanted in black polyethylene bags of 10 L, with a mixture of 30:70 peat moss and tezontle substrate. The management of tomato plants was made according to conventional commercial practices.

Two tunnel-type greenhouses with zenithal ventilation and milky white polyethylene cover were used, one equipped with ventilators to generate the condition of control temperature (CT) and the other with a system of electric heaters to generate the condition of high temperature (HT) and thus obtain a difference in the diurnal temperature (7:00 to 19:00 h), maintaining a similar nocturnal temperature in both treatments. The maximum average temperature was 36.5 °C for HT and 31.5 °C for CT. The air temperature within the greenhouses was recorded every 10 min with Hobo[®] sensors (Onset Computer Corporation, USA).

The quality evaluation of the seeds was made in fruits from the fourth floral cluster. The plants remained in HT and once this cluster reached anthesis, the following seven treatments were established with n=10 plants: 1) plants growing permanently in HT; 2) plants transferred to HT between 5 and 12 daa from the 4th cluster and then returned to HT; 3) plants taken to HT at between 12 and 24 daa; 4) plants taken to HT at between 24 and 36 daa;

5) plants taken to HT at between 36 and 48 daa; 6) plants taken to HT at between 48 and 60 daa; and 7) plants taken to HT at 60 daa and until maturity. The treatments were established, considering an approximate duration of the fruits from the 4th cluster until their maturity at 72 daa (Biais *et al.*, 2014).

Fruit harvesting was carried out in maturity degree 6 (more than 90% of the surface of the fruit is red) (Wan *et al.*, 2018). Seed extraction was made manually from n=20 fruits chosen randomly, through fermentation of the mucilage, then dried at room temperature and forming a single seed lot that was kept at room temperature for 60 d.

Variables

The number of seeds per fruit (NSF) was counted at the time of dissecting the fruit. After the seed storage period, laboratory trials were carried out; the moisture content of the seed was determined in a sample of 0.5 g of seed dried in a conventional Thelco (USA) stove at 103 °C during 17±1 h, which together with the initial weight of the sample was used to calculate the moisture content of the seed lot (expressed in %).

The weight of one thousand seeds (SW, in g) was obtained with the standard procedure by ISTA (2012); four repetitions were used made up of eight samples of 100 seeds, which were weighed in an Ohaus scale (Pine Brook, China), with accuracy of 0.001 g, multiplying the average by 10. The seed length (SL) and width (SW) were measured in four repetitions of 100 seeds, through the processing of images with the Image J[®] software; for this purpose, the seeds were scanned in a multifunctional HP2200 printer (Hewlett Packard, USA) capturing images of 1200 dpi, and both variables were expressed in mm.

For the germination test, four repetitions of 100 seeds were used, which were sown in Petri dishes with moistened filter paper and placed in a SD8900 germinator (Seedbuero Inc., USA) at 25±1 °C for 14 d (ISTA, 2012). The percentage of germination (G, %) was calculated taking into account the number of normal, healthy seedlings and without malformations.

The seed vigor was evaluated with an accelerated ageing test; four repetitions of 100 seeds were used, which were subjected to 41 °C and relative humidity of 100% during 72 h, and after this time the seeds were evaluated in a germination test (GAA).

Electric conductivity (EC, $\mu\text{S cm}^{-1} \text{g}^{-1}$) of the imbibition solution was determined using four repetitions of n=20 seeds that were previously weighed and placed in 20 mL of deionized water at 25 °C for 24 h; after this time, the EC of the solution was measured with a Model 72729 conductometer (Oakton, Singapur). The respiratory rate of the seed during germination was also evaluated (Resp, $\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$), which was measured in four repetitions of 20 seeds with 5 d of imbibition with a CI-301PS photosynthesis meter (CID Inc., Canada).

Experimental analysis

A completely randomized experimental design was used. The means comparison test was carried out with Tukey's method ($P \leq 0.05$). The statistical analyses were made with the Infostat statistical program version 2016e (Universidad de Córdoba, Argentina). To normalize the data of the variables that were recorded in percentage, they were transformed with the following formula: $\text{Arcsine } \sqrt{x} / 100$.

RESULTS AND DISCUSSION

The moisture content of the seeds from the treatments evaluated had an average of 7.6±0.1%, value that does not interfere with the rest of the physical, physiological and biochemical variables because in dry seeds, as is the case, the biological processes are slower than in moist seeds (Ayala-Villegas *et al.*, 2014; Taylor, 2020). After the harvest, the moisture content of the seed decreases until reaching a dynamic equilibrium with the environment surrounding it (Rodríguez-Burgos *et al.*, 2011).

Physical quality of seeds

The weight of one thousand seeds (SW), the seed length (SL) and the number of seeds per fruit (NSF) presented significant differences ($P \leq 0.05$) caused by the treatments studied. Only in the variable seed weight (SW) the differences caused by the treatments were not statistically significant (Table 1).

The seeds produced by treatment seven were showing less weight (2.99 g) compared to the rest of the treatments (Figure 1A), only treatment four was statistically similar; reductions of 6.3 and 4.1% were found, respectively, compared to the highest value (Treatment 1, 3.19 g). Treatments one to six produced seeds with statistically similar weight, the average of SW of these six treatments was 3.14 g. The SW values obtained in this study are similar to those reported by Delgado-Vagas *et al.* (2018) for var. Moneymaker. According to Hampton *et al.*

Table 1. Mean squares and statistical significance in the analysis of variance of the physical quality variables of the Moneymaker seeds produced under seven high temperature conditions

SV	1000-seeds weight (g)	Seed length (mm)	Seed width (mm)	Number of seeds
Treatment	0.02 *	0.22 *	0.14 ns	1246.21 *
Error	0.0026	0.0900	0.0800	0.0024
CV	1.65	10.66	8.28	21.81

SV=source of variation; CV=Coefficient of variation; **=highly significant ($P \leq 0.01$); *=Significant ($P \leq 0.05$); ns=Not significant.

(2013), the increase in temperature can reduce the mass of the seed due to acceleration in the growth rate and reduction in the time of seed filling, and this was probably the reason why the seed did not reach higher weight in treatment seven, whose fruits were subjected to high temperature between 60 daa and fruit maturity, that is, in the last period of their development. The SW values had a positive correlation (R) with those of SL of 0.83* (Figure 1A and 1C), indicating that the increase in SW was mainly determined by a greater growth in seed length.

Treatment five caused the lowest number of seeds per fruit (120) and was statistically similar to treatments six and seven (Figure 1B). The NSF average of treatments

one to four, whose values were statistically similar, was 169 seeds fruit⁻¹. These values were found within the segment of values found by Delgado-Vagas *et al.* (2018).

Physiological quality of seeds

When it comes to physiological quality, it was found that germination (G) and germination after subjecting

the seeds to accelerated ageing (GAA), variable that is considered an expression of seed vigor (Ayala-Garay *et al.*, 2018), showed highly significant differences ($P \leq 0.01$) as an effect of the treatments studied. In the variable electric conductivity of the imbibition solution (EC) and respiration of the seed during germination (Resp), only significant differences were found ($P \leq 0.05$; Table 2).

The values with maximum germination were observed in treatments 1, 2, 3, 4 and 7. Treatment five presented the lowest values of germination and vigor (expressed by the variable GAA) of seeds, with 79.4 and 39.5%, respectively (Figures 2A and 2B). In both variables the values increased immediately after, in treatments 6 and 7.

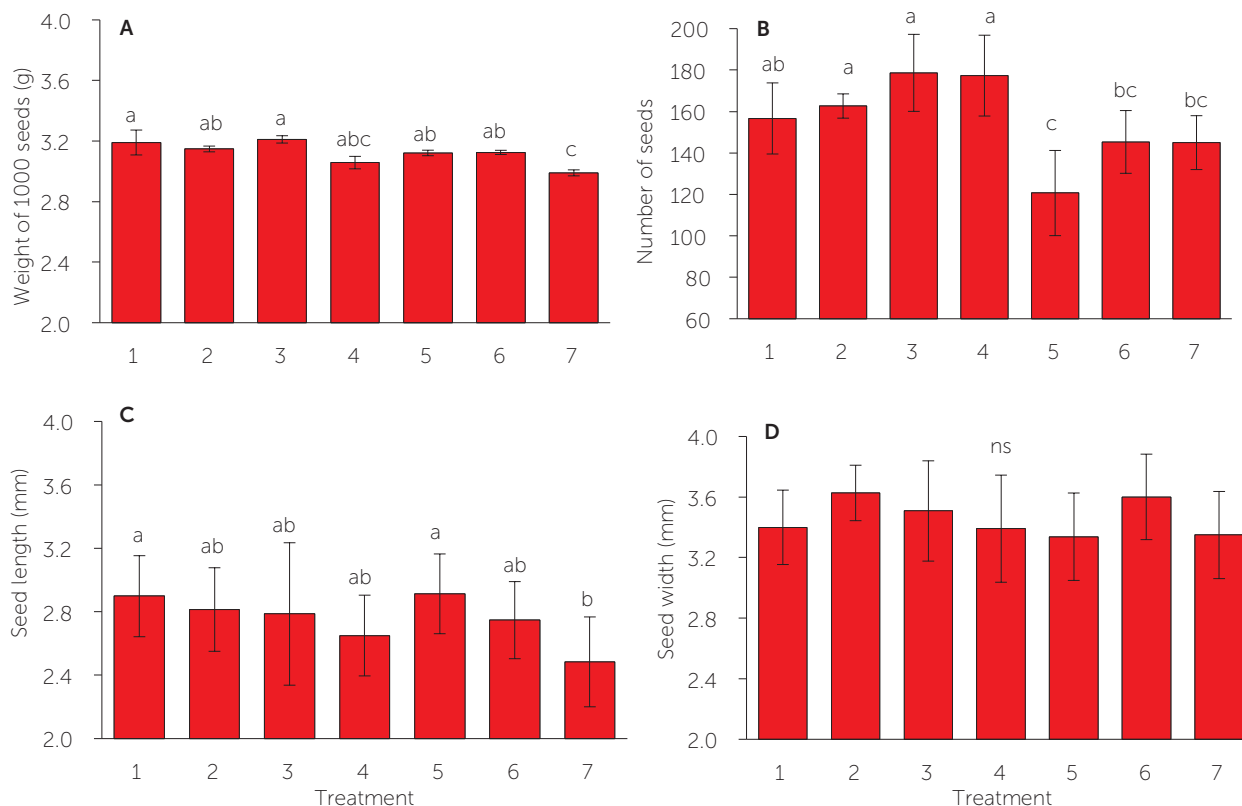


Figure 1. (A) Weight of 1000 seeds (B) Number of seeds per fruit, (C) Seed length (D) Seed width of the seeds of Moneymaker tomato variety produced under seven high temperature conditions. The bars indicate the standard error (n=4). Small letters and "ns" indicate statistical significance between means (Tukey, $P \leq 0.05$).

Table 2. Mean squares and statistical significance in the analysis of variance of the physiological quality variables of the Moneymaker seeds produced under seven high temperature conditions.

SV	Germination (%)	Germination after AA (%)	Electric Conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$)	Respiration ($\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$)
Treatment	0.06 **	0.16 **	52.62 *	38.13 *
Error	0.0100	0.0100	1.300	0.117
CV	5.76	13.68	14.30	30.65

SV=source of variation; CV=Coefficient of variation; AA=Accelerated ageing; **=Highly significant ($P \leq 0.01$); *=Significant ($P \leq 0.05$); ns=Not significant.

Accelerated ageing decreased seed germination of all the treatments, going from 94% in seeds without ageing to 67% average in aged seeds. Since vigor can be positively correlated with seed deterioration, the capacity of a seed lot to survive artificial ageing is correlated with its vigor and potential of longevity in storage (Bewley et al., 2013).

The EC test is used as an indicator of the physiological quality in seeds (ISTA, 2012). When measuring the electric conductivity of the imbibition solution, the solutes liberated by the seeds are measured, which is inversely proportional to their germinability and vigor, since it is considered that a higher EC indicates that the

organization or integrity of the membranes is deficient. In this experiment, the variable EC did not represent a useful element for the analysis, since when the highest values of germination and vigor (treatment one, for example) and when the lowest (treatment five) were found, the values of EC were statistically lower than the group, which is inconsistent with countless authors (Martínez-Muñoz et al., 2019).

Another variable related to seed vigor is respiration, which is controlled by the quantity of respirable substrates such as carbohydrates (Bewley et al., 2013). In this study, respiration of the seed on the fifth day of imbibition (Resp) had a similar behavior in G and

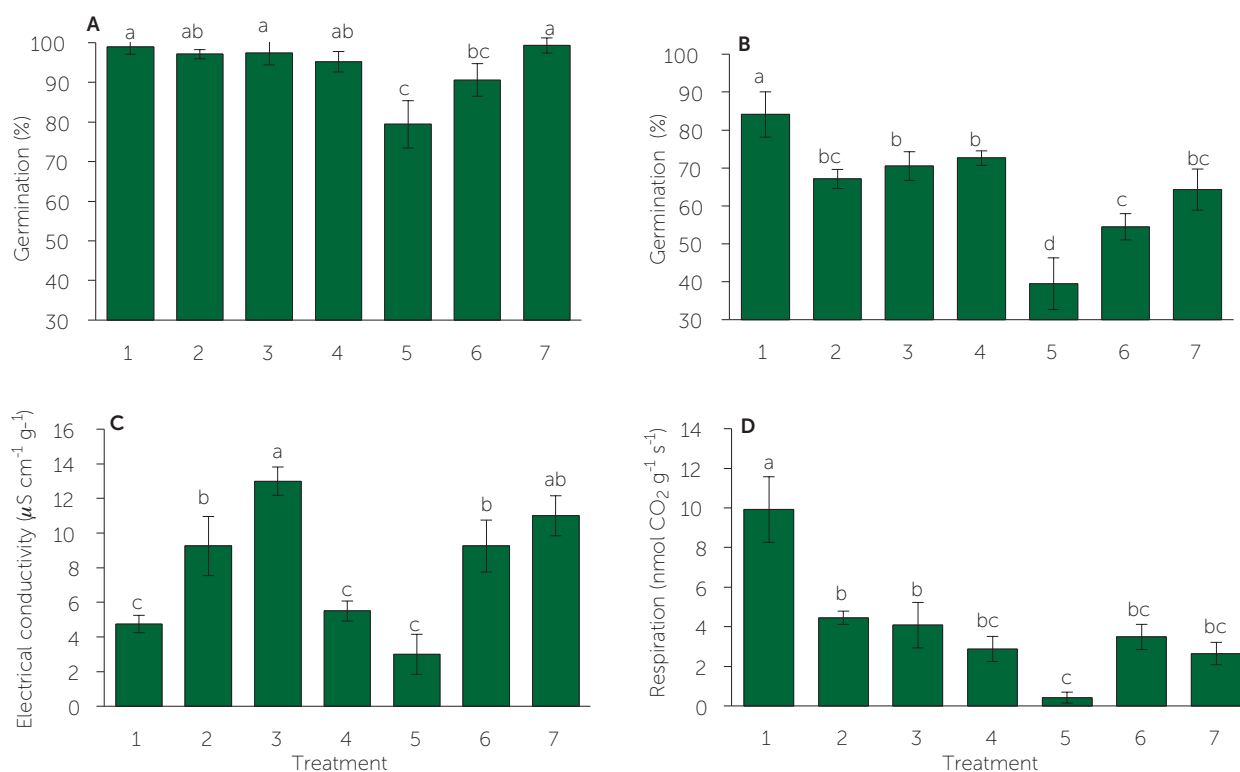


Figure 2. (A) Germination, (B) Germination after accelerated ageing, (C) Electrical conductivity of the imbibition solution, and (D) Seed respiration rate at the fifth day after started germination in the seeds of Moneymaker tomato variety produced under seven high temperature conditions. The bars indicate the standard error ($n=4$). Small letters indicate statistical significance between means (Tukey, $P \leq 0.05$).



GAA, since the lowest value was observed in treatment five. Seed respiration provides energy required for the germination (Pérez-Camacho *et al.*, 2008) and its intensity depends on the functionality of mitochondria (Bewley *et al.*, 2013). Therefore, vigorous seeds require higher energetic contribution than non-vigorous ones (Pérez-Camacho *et al.*, 2008). In this study, the value of the correlation between G and Resp was 0.62* and between GEA and Resp it was 0.81* (Figure 2). The magnitude of the values of Resp recorded in this study is similar to what was observed by Delgado-Vargas *et al.* (2018) in tomato seeds.

CONCLUSIONS

The maximum average temperature of 36.5 °C applied since 60 daa and until fruit harvest resulted in seeds with lower weight and less length. When the tomato fruits were placed at between 36 and 48 daa at this temperature, a lower number of seeds per fruit, germination, respiration value of the seed during germination, and vigor were found. Thus, the high temperature affects the second phase of fruit development provoking lower physical and physiological quality of the seed.

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