Article

Physiological response of ancho pepper seeds (*Capsicum annuum* L.) to growth regulators

Pilar Espitia-Hernández[1§]
Norma Angélica Ruíz-Torres[1]
Mario Ernesto Vázquez-Badillo[1]
David Sánchez-Aspeytia[2]

[1]Center for Training and Development in Seed Technology-Autonomous Agrarian University Antonio Narro. Calzada Antonio Narro num. 1923, Buenavista, Saltillo, Coahuila, Mexico. CP. 25315. Tel. 01 (844) 4110200, ext. 2377. (n_nruiz@hotmail.com; marioe.vazquez@hotmail.com). [2]Experimental Field Saltillo-INIFAP. Highway Saltillo-Zacatecas km 342+119, num. 9515, Hacienda de Buena Vista, Saltillo, Coahuila, Mexico. Tel. 01(55) 38718700. (aspeytia.david@inifap.gob.mx).

Corresponding author: pilar.espitia1@gmail.com.

Abstract

The physiological attributes of pepper seeds are reduced from their development in the mother plant or as a result of physiological mechanisms that reduce germination and cause loss of vigor and viability. The objective of the present study was to evaluate the effect on the germination of ancho pepper seeds imbibed in the following treatments: KNO_3 at 2%, 4% and 6%, promoter I at 0.5%, 1% and 1.5%, promoter II to 1 %, 2% and 3% and the control in distilled water. 120 seeds were weighed to submit them to each treatment for 48 h and obtain the imbibition curves. For the germination test in the laboratory, the conditioned seeds were sown between anchor paper and the evaluation was made after 21 days. The statistical analysis of data was performed in a completely randomized design, and the means were compared with the Tukey test ($p\leq 0.05$). The results showed that the solution of the treatments did not influence the process of imbibition of the seed. The highest percentage of germination was obtained with seeds imbibed in promoter I to 1.5% that reached 90% of germination, 56% more in comparison with the control that was 34%. The promoter I for its content of aminoacids, vitamins, macro and micronutrients, acted as a stimulator of germination.

Keywords: germination, imbibition, promoter.

Reception date: March 2019
Acceptance date: June 2019

Introduction

At the international level, Mexico occupies the eighth place in world production of dried pepper, with 119 958 tons per year and a harvested area of 68 860 ha (SIAP, 2017). Guanajuato is the state that contributes 60% of the country's ancho pepper production, which allocates around 5 700 ha for the production of pepper, of which around 4 000 ha are ancho pepper (Cortez *et al*., 2011).

The production of seedlings in seedlings is a very common practice among producers, it is carried out in order to protect the seedlings from the cold and dispose of them once the frost season is over, so it is necessary to use technologies and greenhouses for the production of quality seedlings, which generates a cost for farmers (Reveles *et al*., 2012). The seedling must comply with the following attributes of quality: good health, well-developed root system, dark green color in the foliage and vigorous appearance (Jasso and Martínez, 2003). With respect to horticultural species, the vigor test is carried out in its initial phase in comparison with other crops of economic interest.

The most commonly used indicator in vegetable seeds to evaluate vigor is the first count in the germination test (Barros and Minami, 2000). The concept of germination is defined as the emergence and development of essential structures from the embryo, which manifest the ability of the seed to produce a normal plant under favorable conditions (Moreno, 1996). The process of germination begins with imbibition and ends with emergence. Imbibition is a phase of water absorption by the seed and emergence refers to the process by which the embryonic axis or radicle grows and passes through the structures that surround it (Azcon and Talon, 2008). Once the radicle begins to elongate outside the seed coat, the germination process has concluded (Carranza *et al*., 2016).

There are commercial products recommended to promote the development of plants, the promoter I used in this research, contains phytohormones of the three main groups: gibberellins (gibberellic acid, 20 ppm), auxins (indole acetic acid, 63 ppm), cytokinins (zeatin, 210 ppm), as well as: carbohydrates (glucose and fructose, 0.7%), vitamins (niacin 0.0006% and inositol 0.43%), nitrogen (1%), phosphorus (0.5%), potassium (4.5%), manganese (0.12%), iron (0.49%) and zinc (0.37%), in addition to being suggested as a synergist of the activity of auxins and cytokinins, with effects on the increase of aerial and root biomass; in fruit vegetables, 1 to 2 L ha^{-1} is recommended (Arysta, 2015a).

Promoter II has extracts of plant origin, it is used as a hormonal phytoregulator whose composition contains three phytohormones: cytokinins (zeatin, 94 ppm), gibberellins (36 ppm), auxins (36 ppm); Other compounds that it includes are: magnesium (2.53 g L^{-1}), sulfur (6 g L^{-1}), boron (3.3 g L^{-1}), iron (5.39 g L^{-1}), manganese (1.32 g L^{-1})), zinc (4.07 g L^{-1}), within its functions is to stimulate various metabolic and physiological processes of plants, such as: cell division and differentiation, substance translocation, chlorophyll synthesis and differentiation of buds, among others, the recommended dose for pepper is 450 to 500 mL ha^{-1} (Arysta, 2015b).

With respect to KNO_3, one of its main functions is that it favors the metabolic repair of tissues and the increase of respiration; therefore, it improves the rate of growth and germination (Shim *et al.*, 2008). The latter and the content of the first two products have been used in the conditioning of the seeds, in order to reinvigorate, accelerate and standardize the germination of the seeds of the *Capsicum* genus (Garruña *et al*., 2014). Therefore, the objective of the present study was to evaluate the physiological quality of ancho pepper seeds imbibed in different solutions of growth regulators.

Materials and methods

Genetic material

San Luis variety ancho pepper seeds, packed on February 16, 2017.

Treatments

Three products were used: $KNO_3 \geq$ 99.6% (2, 4 and 6% w/v), promoter I (0.5, 1 and 1% v/v), promoter II (1, 2 and 3% v/v) and as control distilled water for imbibition of the seeds. All the products were diluted in distilled water.

Location

The tests were carried out in the Seed Physiology Laboratory of the Center for Training and Development in Seed Technology (CCDTS), of the Autonomous Agrarian University Antonio Narro (UAAAN), which is located between the geographic coordinates of 25° 22’ of latitude north and 101° 02’ west longitude and at an altitude of 1 742 m.

The experiment was carried out in two stages

Stage I. Imbibition curves

To determine the imbibition curve, the weight of 120 seeds was taken on an Ohaus analytical balance with a precision of 310 g x 0.001 g, then submerged in 20 ml of distilled water for the controls and 20 mL in solution of each treatment, for this, 40 mL beakers were used. During the first 12 h, every 2 h, the seeds were drained in plastic strainers, dried with absorbent paper towels and weighed again; this activity was repeated every 12 h until the end of the process at 48 h (0, 2, 4, 6, 8, 10, 12, 24, 36, 48 h). The volume imbibed (mL) of the treatments by the seeds, was determined with the difference of weight between the initial weight and the final weight, the imbibition of the units of weight was calculated, transforming them to units of volume (on the basis of which 1 g of increased weight= 1 mL imbibed).

Stage II. Germination tests between paper

After 48 hours, the seeds were sown between anchor paper moistened with distilled water, 4 repetitions per treatment of 25 seeds each, rolled into a cue and placed vertically in plastic bags inside a Thermo Cientific bioclimatic chamber (Precision) at a controlled temperature of 25 ±2

°C and photoperiod of 16 h light/8 h of darkness (ISTA, 2016). Germination was evaluated at 21 days by quantifying percentage of: normal seedlings (PN), abnormal seedlings (PA) and ungerminated seeds (SSG), in addition to the plumule (LP) and radicle (LR) lengths, which were measured with a millimeter paper template and the mean per repetition of each treatment was determined.

The percentage of normal seedlings (PN) was considered as the total of those whose essential structures were well developed under favorable conditions of water, light and temperature (Moreno, 1996), were counted and divided between the total of seeds planted between paper, in the same way the percentage of abnormal seedlings (PA) and ungerminated seeds (SSG) was obtained.

For the study of the variables the statistical package Statistical Analysis System (SAS) version 9.0 (SAS Institute, 2002) was used, with the data an analysis of variance was carried out (Andeva). The mean values were submitted to the Tukey test $p \leq 0.05$.

Results and discussion

Stage I. Imbibition curves

For the first phase of the germination process, it was observed among the seeds, that the highest imbibition rate occurred during the first 2 h (Figure 1a and 1b), raising its initial dry weight to 46%; after 2 h, the increase in weight was slower as time passed. After 24 hours, the second phase of the germination process was reached, registering an increase of 78% with respect to its dry weight (Figure 1b). In this second stage of germination, a constant behavior was observed in the hydration of the seeds, there was no emergence of radicle in any of the evaluated treatments, that is to say, no phase III was reached.

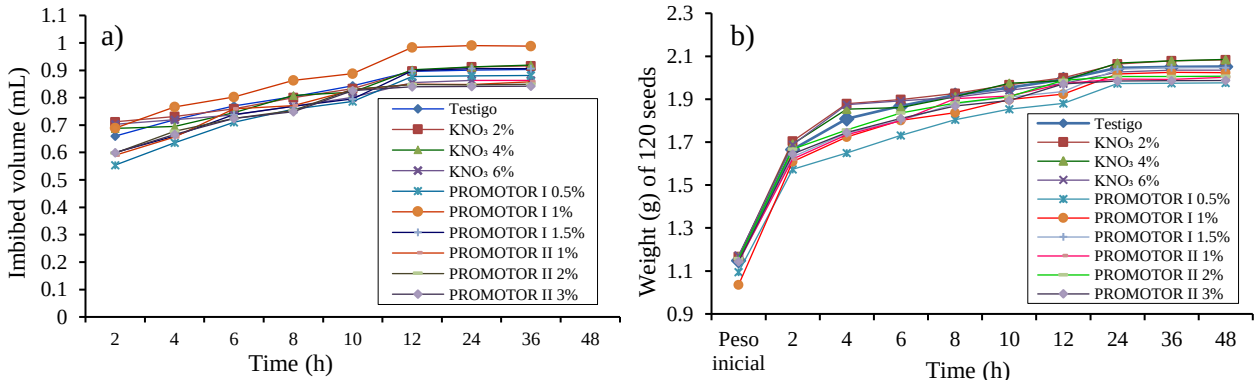


Figure 1. a) imbibed volume (mL) of 120 ancho pepper seeds during 48 h, in nine treatments; b) imbibed weight (g) of 120 ancho pepper seeds during 48 h, in nine treatments.

This is attributed to the fact that the water capacities of the seed and of the solution come into equilibrium, so that the metabolic processes stop when liquid is not introduced into the seed (Akers and Holley, 1986), which prevents the radicle emergency occurs. Sousa *et al*., (2006) reported that during the process of imbibition of *Swietenia macrophylla* seeds, the increase in the weight of the seed was greater, with respect to the volume, which was discrete. In this experiment the same

behavior was observed in the imbibition curves for the first two phases of the germination process; that is, the first of rapid absorption of moisture and the second stationary phase that was characterized at the end, by the absence of solution absorption of the treatments (Figures 1a and 1b), without reaching stage III.

The analysis of variance did not show significant differences ($p \leq 0.01$), for the increase in grams of weight in the seeds and the volume imbibed (mL) of solution between the treatments (Table 1); this is due to the fact that the rates of absorption of solution in the seeds exposed to the different treatments were uniform, which indicates that the type of conditioning solution of the treatments did not influence the water disposition by the seed.

Table 1. Mean squares for weight and volume of solution imbibed by ancho pepper seeds.

FV	GL	Imbibed weight (g)	GL	Imbibed volume (mL)
Treatments	9	0.015 NS	9	0.0104 NS
Error	90	0.02	80	0.017
Total	99	0.07	89	
CV	14.89%		17.25%	

FV= sources of variation; GL= degrees of freedom; CV= coefficient of variation; NS= not significant at $p \leq 0.01$.

Stage II. Germination tests between paper germination

According to the analysis of variance, in all the variables: percentages of normal seedlings (%PN), abnormal seedlings (%PA), ungerminated seeds (%SSG), plumule length (LP) and length of radicle (LR) significant differences were obtained ($p \leq 0.01$) between treatments (Table 2).

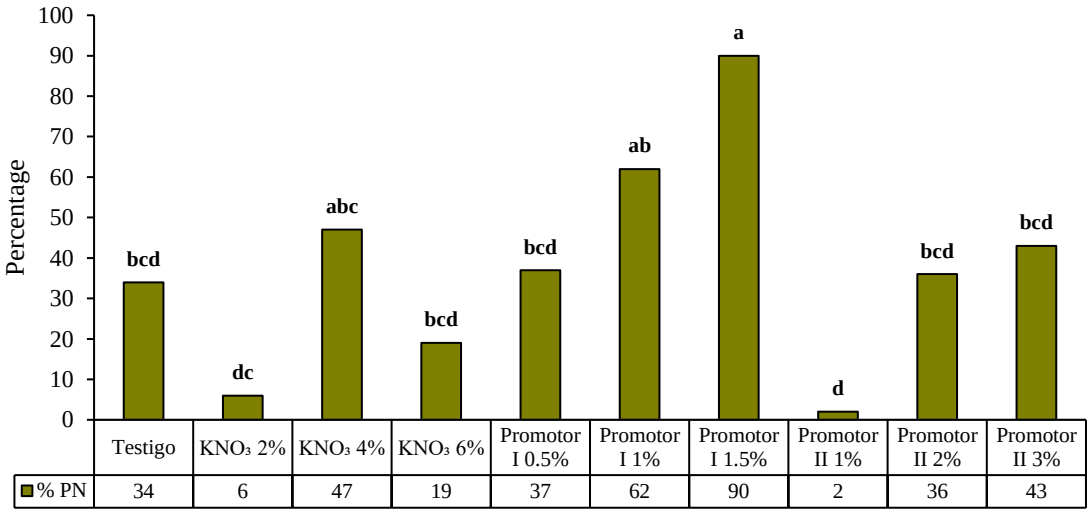
Table 2. Mean squares for percentage of normal seedlings (PN), abnormal seedlings (PA), ungerminated seeds (SSG), plumule length (LP) and radicle length (LR).

FV	GL	PN (%)	PA (%)	SSG (%)	GL	LP (cm)	LR (cm)
Treatments	9	2705.07**	1724.67**	2291.96**	9	8.13**	107.98**
Error	30	322.13	292.4	111.33	369	0.61	4.83
Total	39				378		
CV		47.73%	43.29%	46.08%		29.38%	20.42%

**= significant at $p \leq 0.01$; FV= sources of variation; GL= degrees of freedom; CV= coefficient of variation.

It is important to mention that due to the possible deterioration that the seeds presented, the percentage of normal seedlings had variable responses that is confirmed by observing the result of the control (34%), with respect to the KNO_3 treatments at 2 and 6% and the promoter II at 1%. The treatment with promoter I at 1.5% showed a better percentage of normal seedlings (Figure 2), with 90%, which showed an increase of 56% with respect to the control. This result is attributed to the concentrations of phytohormones and microelements, whose positive effect is expressed with the increase in the percentage of germination, which agrees with the gibberellic acid that could have stimulated in the seeds, the synthesis of the starch for its cleavage in sugars more simple ones such as glucose, which were available as an energy source for the cells of the embryo during the

germination process; likewise possibly the concentration of auxins and cytokinins (zeatin) had stimulating effects on the growth of stems and roots and in general in the formation of seedlings, since they promote cell elongation.



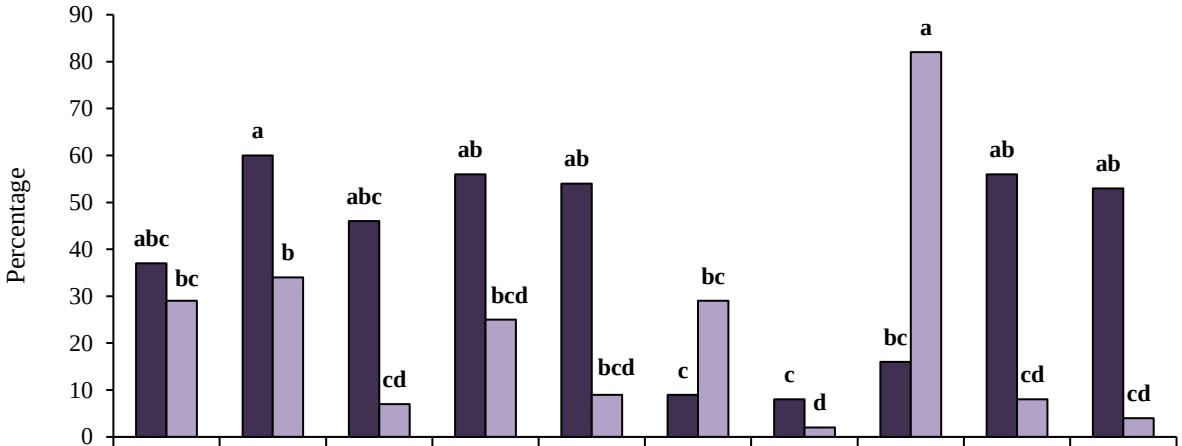
Means with the same letter are not significantly different (Tukey, 0.05)

Figure 2. Comparison of means in percentage of normal seedlings (%PN).

With the 4% KNO_3 (47% of PN) a greater number of normal plants was obtained with respect to the concentrations of 2% (6% of PN) and 6% (19% of PN), which coincides with the reported with Garruña *et al*. (2014) who evaluated the effect of a concentration close to 3% on the conditioning of seeds of habanero pepper and obtained a positive effect in the emergence of seedlings (91% of PN) surpassing the control (72% of PN).

The seeds imbibed with KNO_3 at 2% obtained the highest percentage in abnormal seedlings with 60% (Figure 3), this result could be due to the concentration of the treatment, in addition to the effects of potassium nitrate on the process of germination in pepper seeds (Andrade and Laurentin, 2015). The treatment with the highest percentage in ungerminated seeds was obtained with the promoter II at 1% with 82% (Figure 3), this result can be attributed to the concentration, since in normal seedlings at 2 and 3% numerically exceeded the control, this increase agrees with the results obtained by Gonzalez *et al*. (2015) who with the same promoter at 1.6% and during 24 h of immersion of amashito pepper seed (*Capsicum annuum* L. var. *Glabriusculum*), significantly increased the germination up to 86%.

In relation to plumule length (LP), according to Tukey, significant differences were obtained ($p \leq 0.05$), in Figure 4 it is observed that the KNO_3 treatment at 4% was statistically better with 3.69 cm, the previous coincides with the exposed by Shim *et al*. (2008), mention that this product favors the metabolic repair of tissues and the increase of respiration, which improves the rate of growth and germination. The treatments were followed: KNO_3 at 2% with 3.50 cm, KNO_3 at 6% with 3.28 cm, promoter II at 1% with 3 cm, compared to the control which was 2.74 cm. For the variable length of radicle (LR) in Figure 4 it can be seen that the promoter II at 1% was statistically better at 14 cm.

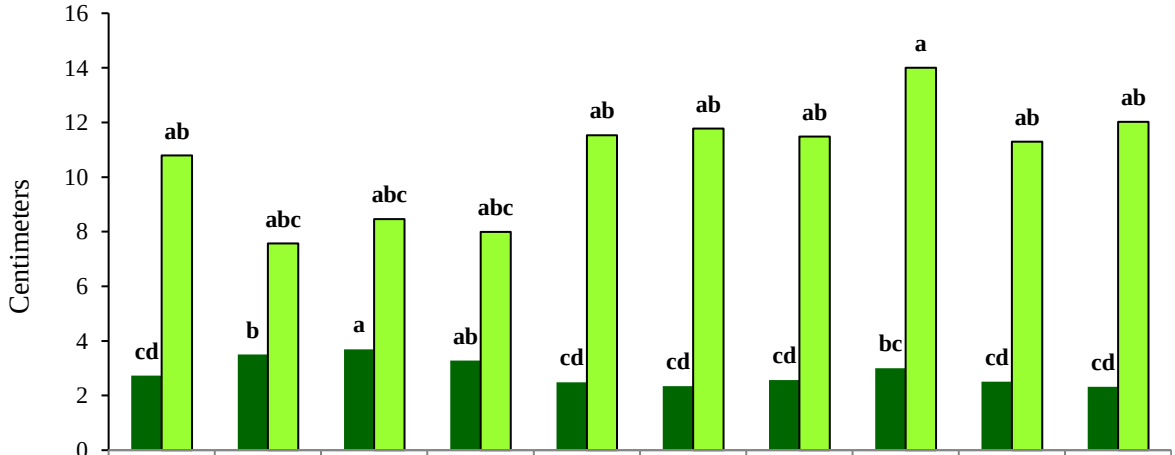


	Testigo	KNO_3 2%	KNO_3 4%	KNO_3 6%	Promotor I 0.5%	Promotor I 1%	Promotor I 1.5%	Promotor II 1%	Promotor II 2%	Promotor II 3%
% PA	37	60	46	56	54	9	8	16	56	53
% SSG	29	34	7	25	9	29	2	82	8	4

Means whit the same letter are not significantly different (Tukey, 0.05)

Figure 3. Comparison of means in percentage of abnormal seedlings (% PA) and non-germinated seeds (% SSG).

Although they were statistically equal to the control, numerically exceeded, the treatments: Promoter II 3% with 12.03 cm, Promoter I with 1% with 11.77 cm, 0.5% with 11.53 cm, 1.5% with 11.48 cm and promoter II with 2% with 11.29 cm. It is important to note that although the latter at 1%, it was statistically similar to the control, this treatment obtained the lowest percentage of normal seedlings (2%) (Figure 4).



	Testigo	KNO_3 2%	KNO_3 4%	KNO_3 6%	Promotor I 0.5%	Promotor I 1%	Promotor I 1.5%	Promotor II 1%	Promotor II 2%	Promotor II 3%
LP (cm)	2.74	3.50	3.69	3.28	2.48	2.34	2.57	3.00	2.51	2.32
LR (cm)	10.79	7.57	8.46	7.99	11.53	11.77	11.48	14.00	11.29	12.03

Means with same letter are not significantly different (Tukey, 0.05)

Figure 4. Comparison of means in length of plumule (LP) and length of radicle (LR).

Conclusions

According to the results, the rate of imbibition of all the treatments was the same in the pepper seed of the San Luis variety. It is concluded that the promoter I at a concentration of 1.5% (v/v) and 1% (v/v), have a positive effect on the increase of germination in relation to normal seedlings (90% and 60%, respectively) of ancho pepper, as well as the good development of the radicle, which is important for the establishment of plants in the field.

In relation to the length of plumule, the best treatment was KNO_3 at 4%, which was also one of the treatments that exceeded the control in terms of percentage of normal seedlings.

Acknowledgments

To the Center for Training and Development in Seed Technology of the Autonomous Agrarian University Antonio Narro and to the Saltillo Experimental Field of the National Forestry, Agriculture and Livestock Research Institute, for the support in carrying out the project: response to the application of growth regulators plant on the germination of seeds of low vigor, development and establishment of plants of ancho pepper and serrano pepper (*Capsicum annuum* L.). In this article we present a part of the results of the first phase of the experiment.

Cited literature

Akers, S. and Holley, K. 1986. SPS: A system for priming seed using aerated polyethylene glycol of salt solutions. HortSci. 21:529-531.

Andrade, S. y Laurentin, H. 2015. Efecto del nitrato de potasio sobre la germinación de semillas de tres cultivares de ají dulce (*Capsicum chinense* Jacq.). Rev. Unellez de Ciencia y Tecnología 33:25-29.

Arysta LifeScience. 2015a. Bioestimulante Fitobolic®. http://arysta.cl/arystahome/wp-content/uploads/2018/08/ft_fitobolic-v011-21-08-2018-1.pdf.

Arysta LifeScience. 2015b. Biozyme TF, extractos de origen vegetal 78.87 % L.

Azcón, B. J. y Talón, M. 2008. Fundamentos de fisiología vegetal. 2ª. Edición. McGraw-Hill Interamericana. Barcelona España. 656 p.

Barros, T. S. y Minami, K. 2000. Qualidade fisiológica de sementes de pimentão. Scientia Agrícola 57(1):109-112.

Carranza, C.; Castellanos, G.; Deaza, D. y Miranda, D. 2016. Efecto de la aplicación de reguladores de crecimiento sobre la germinación de semillas de badea (*Passiflora quadrangularis* L.) en condiciones de invernadero. Rev. Colomb. Cienc. Hortíc. 10(2):284-291.

Cortez, E.; Rivera, J. G.; Andrio, E.; Guevara, R. G.; Guevara, L.; Cervantes, F. y Mendoza, M. 2011. Osmoacondicionamiento de la semilla de chile ancho y su efecto en el vigor. Universidad y Ciencia. 27(3):345-349.

Garruña, H. R.; Latournerie, M. L.; Ayala, G. O.; Santamaría, J. M. y Pinzón, L. L. 2014. Acondicionamiento pre-siembra: una opción para incrementar la germinación de semillas de chile habanero (*Capsicum chinense* Jacq.). Agrociencia. 48(4):413-423.

González, C. N.; Jiménez, V. R.; Guerra, B. E. C.; Silos, E. H. y Pairo C. E. 2015. Germinación del chile amashito (*Capsicum annuum* L. var. *Glabriusculum*) en el sureste mexicano. Rev. Mex. Cienc. Agríc. 11:2211-2218.

ISTA. 2016. International Seed Testing Association. Introducción a las reglas ISTA capítulos 1-7, 9. Reglas Internacionales para el Análisis de las Semillas. The International Seed Testing Association. Bassersdorf, Suiza. 192 p.

Jasso, Ch. C. y Martínez, G. M. A. 2003. Guía para la producción de chile ancho con fertirriego y acolchado plástico en el altiplano de San Luis Potosí. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). San Luis Potosí, México. Folleto para productores núm. 38.

Moreno, M. E. 1996. Análisis físico y biológico de semillas agrícolas. 3ª (Ed.) Universidad Nacional Autónoma de México (UNAM). México, DF. 393 p.

Reveles, H. M.; Huchín, A. S. y Velásquez, V. R. 2012. Producción de plántula de chile en invernadero: manual para el productor. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Zacatecas. Folleto para productores núm. 37. 38 p.

SAS Institute. 2002. SAS User's Guide. Version 9.0, SAS Institute. Inc. Cary, NC. USA.

Shim, S. I.; Moon, J. C.; Jang, C. S.; Raymer, P. and Kim, W. 2008. Effect of potassium nitrate priming on seed germination of seashore paspalum. HortSci. 43:2259-2262.

SIAP. 2017. Servicio de Información Agroalimentaria y Pesquera. Datos abiertos estadística de producción agrícola. http://infosiap.siap.gob.mx/gobmx/datosAbiertos.php.

Sousa, P. E. A.; Lemos, F. J. P. and Trombert, O. D. M. 2006. Imbibition of *Swietenia macrophylla* (Meliaceae) seeds: the role of stomata. Ann. Bot. 98(1):213-217.