MedCrave
Step into the World of Research

Horticulture International Journal

Research Article

Open Access

CrossMark

Vacuum test and type of substrate on the germination and vigor of seeds of *Gossypium* spp

Abstract

The native Mexican species of *Gossipium* (Cotton) are such important plants, that they deserve to be differentiated by both traits: the seed quality and vigor. For that reason, it was assayed a practical vigor test to establish whether it would be able to depict them with precision. With a chosen vacuum vigor test, the germination capacity and vigor of seeds of G. *hirsutum* and G. *lobatum* were determined using: two types of paper as substrate and environments with oxygen deficiency, given by the vacuum conditions of 300, 500, 600 and 700 mm of Hg to which the seeds were subjected during the germination process (12days at 25±1°C). The statistical meanings resulted in all the variables for the blocks, the substrate and the vacuum conditions, in double and triple interactions ($p<0.0001$, $p<0.005$, respectively). The results of the vigor test showed that the G. *hirsutum* species exhibited the best vigor condition. The cotton seeds in the substrate Versa Pak exhibited the best condition of vigor. The germination and vigor of seeds of these species decreased exponentially as the levels of vacuum increased, which are equivalents to the reduction of oxygen in the respective vacuum levels. In addition, the estimated oxygen thresholds for G. *hirsutum* and G. *lobatum* determined the germination and vigor of the seeds.

Keywords: G. *hirsutum*, G. *lobatum*, lack of oxygen, dry biomass

Volume 3 Issue 3 - 2019

Claudia Pérez-Mendoza,[1] Ma del Rosario Tovar-Gómez,[2] Guillermo Carrillo-Castañeda,[1] Javier Suarez-Espinosa,[1] Gabino García de los Santos[1]

[1]Colegio de Postgraduados, Campus Montecillo, México
[2]INIFAP-Campo Experimental Valle de México, Carretera Los Reyes-Texcoco, México

Correspondence: Guillermo Carrillo-Castañeda, Genética, Colegio de Postgraduados, Campus Montecillo, 56230, Montecillo, Estado de México, Email carrillo@colpos.mx

Received: June 08, 2019 | **Published:** June 17, 2019

Introduction

Mexico is the center of origin of eleven species of cotton (*G. armourianum*, *G. lobatum*, *G. gossypioides*, *G. aridum*, *G. laxum*, *G. schwendimanii*, *G. thurberi*, *G. trilobum*, *G. davidsonii*, *G. turneri* and *G. harknesii*) and a semi-domesticated specie (*G. hirsutum*);[1] the latter, presents different natural colors tones in its fiber, such as white, green or "*coyuche*" brown.

During the last years the advances in the biology of the seeds have allowed to understand better his physiology and in that context, Gil & López,[2] studied the germinative characteristics of seeds of cotton native to Peru of the species *G. hirsutum* with fibers colored green, lilac and brown, and they showed that the variety with the brown fibers registered high percentages of germination (92%) and a high rate of emergence (74.6%), which is associated with a higher degree of domestication, unlike the other varieties, with emergence and germination values were lower; what coincides with an incipient domestication.

Due to this, it is important to characterize and know the quality of the cotton seeds of native Mexican species. There are laboratory tests to know the physiological condition of the seed such as the standard germination test, carried out under ideal conditions of light, temperature, humidity, optimal aeration and substrates, microorganisms free, etc.[3]

In search of protocols to better predict what may occur with seeds in the field, several vigor tests have been designed to evaluate the germination potential of seeds by subjecting them to a stress condition, during germination.[4] McDonald,[5] mentions that a vigor test must be practical, cheap, quantitative, reproducible, and correlated with plantlet emergence on the field. As expected, there is not a universal test to evaluate the condition of vigor of all kind of seeds.[6]

It was explored the strategy of performing a vigor test based on vacuum stress because the lack of oxygen has been considered to be one of the main environmental factors that curbs seed germination.[7] Some authors have discussed the impact of the lack of oxygen up to extreme conditions, on the germination process.[8,9]

It has been reported that wheat, maize and sorghum seeds with O_2 concentrations of 20.9% have been proven to gradually reduce the speed and percentage of germination.[10,11] Seeds with high contents of fatty acids, such as soybeans (Xiao-Hai *et al.,* 2005) or cotton, are considered as species that are intolerant of oxygen deficiency (anoxia)[10,12,13] since they require 21% O_2 to be able to use the reserve compounds in seed germination,[12] whereas *Oryza sativa* L. (Rice) seeds may germinate under high deficiency of O_2 in the environment, since this capability depends on the process of the ethanolic fermentation (Magneshi & Perata, 2009).

On the other hand, the substrate used in the germination tests is of great importance, since factors such as ventilation, structure, water retention capacity, degree of infestation with pathogens, and others, may vary between substrates; which together determine the promotion or inhibition of seed germination.[14]

It is desirable to have a practical tecnique with discriminatory sensitivity to rank types of seeds according to two important functions such as germination and development, both highly dependent on the amount of oxygen in the medium, especially germination, in order to accurately differentiate the species of this genus presents in Mexico, Due to the above, the aim of this investigation was to assay a vigor test, based on vacuum stress and using two kind of substrates, in order

© 2019 Pérez-Mendoza et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and build upon your work non-commercially.

Copyright: ©2019 Pérez-Mendoza et al.

to determine its sensitivity and feasibility to determine the condition of vigor of seeds of two species of *Gossypium* spp.

Materials and methods

The investigation took place in the year 2017 in the Molecular Genetics Laboratory at the Colegio de Postgraduados, Montecillo, Texcoco, Mexico.

Seeds of the wild species natives to Mexico: *G. hirsutum* (brown fiber) and *G. lobatum,* and the types of substrates, brown interfolded paper towels (Mocambo®) and the Versa Pak (also known as Seedburo® K-24), which have contrasting physical and chemical characteristics, were used in the present research.

In Petri dishes, 90 mm in diameter by 14 mm high, the seeds were scattered on the substrate, previously dampened with distilled water, considering, for each species, four repetitions with 10 seeds each and, placed in glass desiccators to submit them to vacuum conditions (VAC) of 0, 300, 500, 600 and 700 mm Hg, according to the methodology proposed by Artola et al.[7] with the modifications indicated in this study.

The seeds, without scarifying, were kept for 12 days under the vacuum conditions at the laboratory conditions (25°C). The control treatment was seeds placed in a glass desiccator without applying vacuum. The variables evaluated were percentage of total germination (PTG) and weight of total dry biomass of normal seedlings (WTDBS) expressed in milligrams (mg) after drying in a furnace at 70°C for 72 hours.[3]

For the treatments here studied was used a factorial arrangement in general random blocks design, where the blocks were both species of *Gossypium*, factor A, the types of substrate and factor B, the condition of vacuum and four repetitions. The sample size for the germination test is 50 seeds per repetition, although certain wild species do not produce as much seed and therefore, the use of this sample size is difficult to achieve, in consequence, we proceeded to estimate the affordable optimum size according to the methodology proposed by Muller & Benignus[15] and Castelloe[16] for the experimental design of random blocks with a level of significance of 0.05%. Before the analysis of variance, the data of germinated seeds expressed as percentages were transformed using the function of $T=arcsine=\sqrt{y/100}$, where y is the value to transform and T, the value of the variable transformed. The data of the response variables underwent an analysis of variance using the program Statistical Analysis System (SAS, 2000) version 9.0, and the differences between treatments were estimated using Tukey's test with 5% significance. The information on total germination as a percentage underwent the non-linear regression analysis using the quadratic function.[17]

Results

Statistical significances were recorded in all variables for blocks ($p<0.005$), substrates ($p<0.0001$) and condition of vacuum ($p<0.0001$), as well as, in the double interaction of substrates *x* condition of vacuum ($p<0.0001$) and in the triple interaction of blocks *x* substrates *x* condition of vacuum ($p<0.0001$; $p<0.005$). The coefficient of determination (R^2) ranged from 0.91 and 0.99, values that are close to 1, indicating a good adjustment of the statistical model, to describe the relation between the variables of study; the CV varied between 16.2 and 27.1%.

The germination of seeds of *G. hirsutum* was superior in 13.2% with respect to *G. lobatum*, which exhibited the minimum percentage of total germination (Figure 1). The seedlings of *G. hirsutum*, in addition, had a dry biomass weight superior to *G. lobatum* which meant a percentage variation of 66.1% (Figure 1).

Regarding the type of substrate tested, it was demonstrated that in Versa Pak (Figure 2) the percentage of seed germination was higher than in the brown Sanita paper towel, being 16.1% the difference in germination between substrates.

Regarding the dry biomass accumulated by the total seedlings, in the substrate Versa Pak it was slightly higher (0.40 mg) in relation to the WTDBS in the brown Sanita paper towel (0.34 mg) (Figure 2). These results could perhaps be explained by the differential physical-chemical characteristics of such products, in fact, in previous studies (data not shown), the Versa Pak was characterized by having greater thickness and porosity and better water absorption capacity, that caused an increase in the total germination of seeds and in addition, the seedlings accumulated greater amounts of dry biomass, while the brown Sanita paper towel showed less thickness, porosity and water absorption capacity.

It was observed that as the level of vacuum increased the less germination capacity of the seeds of both cotton species. Under the condition of 300 mm Hg, the germination had a slight reduction, as compared to control seeds caused by the reduction of O_2, at 500 mm Hg the germination plummeted and only 1.57% germination was observed at 700 mm Hg (Figure 3). Likewise, the dry biomass weight of total seedlings decreased as the level of the vacuum condition increased.

On the other hand, the results of the double interaction of substrate and vacuum condition, indicated that the germination rate was higher in the seed scattered on the substrate Versa Pak, in treatments VAC 300 and VAC 500, whereas for the case of brown Sanita towel, germination only at the level of VAC 300 was better according to its PTG (Figure 4).

Regarding the combination of SU x VAC, it was observed that in the combination of brown Sanita towels and the VAC 300 condition, the dry biomass weight of seedlings was slightly higher while the combination of the two substrates with the conditions VAC 300, VAC 500 and VAC 600, the WTDBS were similar, except in treatment VAC 700, which provided no information (Figure 5).

As it may be expected, it was obtained in the condition VAC 0 the highest values of germination and accumulation of dry biomass of seedlings in both cotton species. These results may indicate that under that condition, seeds had no O_2 restriction during the process of germination and development. On the other hand, the germination of the species *G. hirsutum* and *G. lobatum* decreased as the condition of vacuum increased from 0 to 700 mm Hg, expressing the values obtained for the parameters of the function by non-linear regression, as: y = a+bx+cx2, where a=87.3747, b=0.1088 and c=-0.00029, respectively in *G. hirsutum*. *G. hirsutum* exhibited the highest percentage of germination in treatment VAC 300, but it gradually decreased down to 24.64 (VAC 500), 45.66 (VAC 600) and 74.48 (VAC 700) percent units (Figure 6).

In the case of *G. lobatum*, the function of non-linear regression, was expressed as $y=a+bx+cx^2$, where a= 93.5082, b=0.0801 and c=-0.00030, respectively. The highest germination rate was under the condition of VAC 0 and it was decreasing drastically down to 37.39 (VAC 500), 64.10 (VAC 600) and 97.24 (VAC 700) percent units (Figure 6).

Citation: Pérez-Mendoza C, Tovar-Gómez MR, Carrillo-Castañeda G, et al. Vacuum test and type of substrate on the germination and vigor of seeds of *Gossypium* spp. *Horticult Int J.* 2019;3(3):166–171. DOI: 10.15406/hij.2019.03.00125

Copyright: ©2019 Pérez-Mendoza et al.

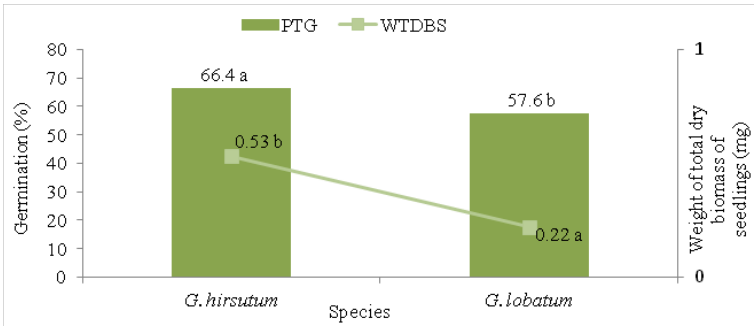

Figure 1 Percentage of total germination (PTG) and total dry biomass weight (WTDBS) exhibited by the seedlings of the species of *Gossypium spp*. Averages with the same letter are statistically equal (Tukey p≤ 0.05).

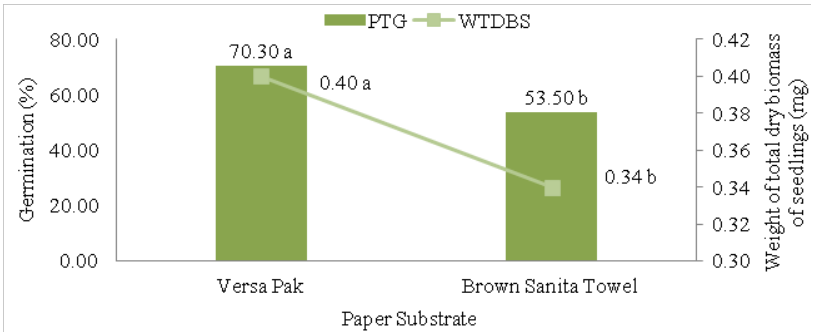

Figure 2 Percentage of total germination (PTG) and weight of total dry biomass (WTDBS) of seedlings developed on the two types of substrate. Averages with the same letter are statistically equal (Tukey p≤0.05).

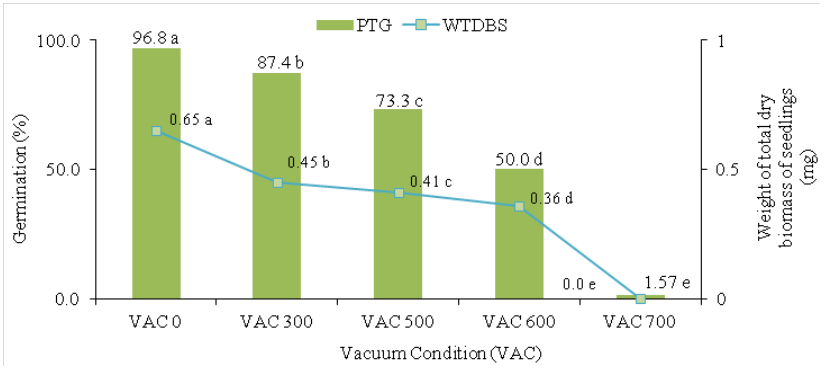

Figure 3 Effect of the condition of vacuum on the percentage of total germination (PTG) and total dry biomass weight of seedlings (WTDBS). Averages with the same letter are statistically equal (Tukey p≤0.05).

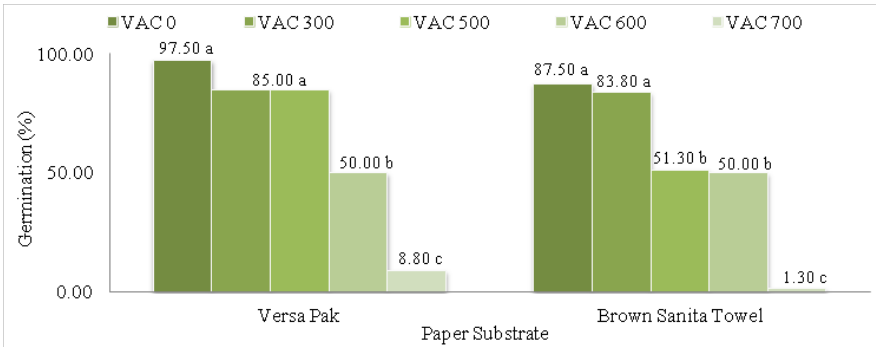

Figure 4 Effect of the combination of substrate and vacuum conditions on the percentage of total germination in the species of *Gossypium* spp. Averages with the same letter are statistically equal (Tukey p≤0.05).

Citation: Pérez-Mendoza C, Tovar-Gómez MR, Carrillo-Castañeda G, et al. Vacuum test and type of substrate on the germination and vigor of seeds of *Gossypium* spp. *Horticult Int J.* 2019;3(3):166–171. DOI: 10.15406/hij.2019.03.00125

Copyright: ©2019 Pérez-Mendoza et al.

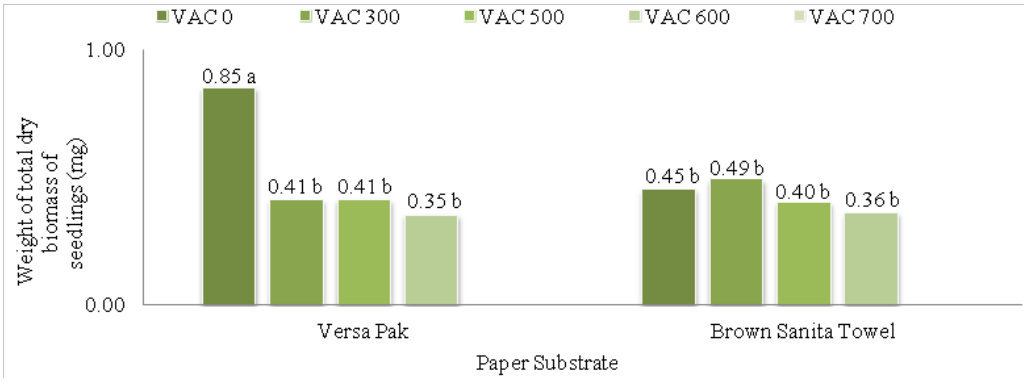

Figure 5 Effect of the combination of substrate and vacuum condition on the total dry biomass weight of seedlings. Averages with the same letter are statistically equal (Tukey p≤0.05).

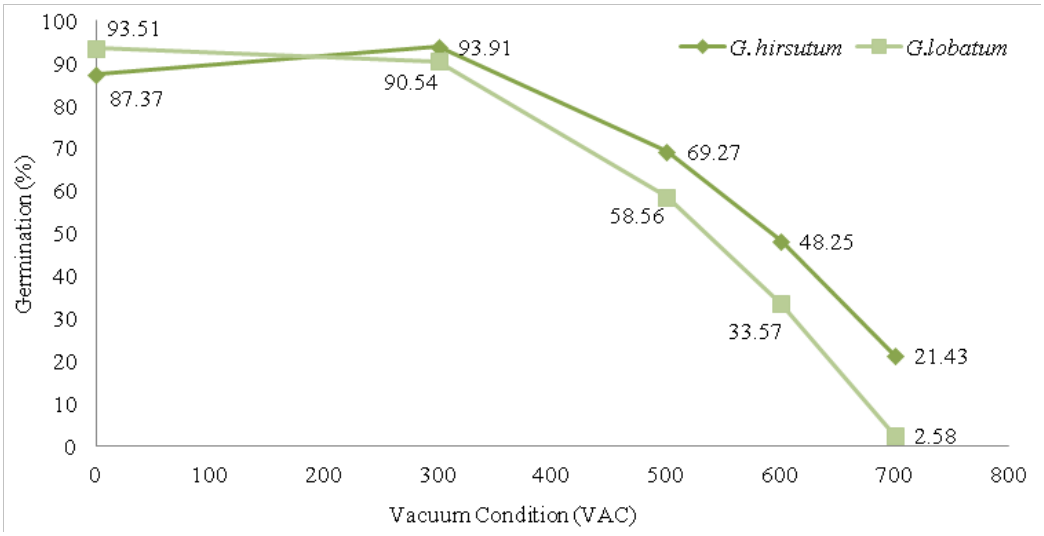

Figure 6 Kinetics of seed germination of *G. hirsutum* and *G. lobatum* under the conditions of vacuum indicated.

Discussion

In order for seeds to germinate, embryo cells require specific amounts of oxygen to carry out the energy-generating processes, such as respiration and fermentation.[18] Both processes imply an exchange of the gases CO_2 and O_2 between cells and the environment[18] in such a way to avoid the phases of germination from being affected, normal atmosphere are required, composed of 21% of O_2 and 0.03% of CO_2.[19]

Oily seeds such as cotton, soybean or sunflower are intolerant or susceptible to the lack of O_2, molecules required for germination. Due to the high amounts of lipids accumulated in their seeds,[12,20] they require higher amounts of O_2 to carry out their metabolic activities, respiration and fermentation.[12] On the contrary, rice seeds in the presence of as low as 0.3% of O_2 are able to germinate at rates of 80% and such resistance to anaerobiosis is linked to the high amount of starch accumulated in their seeds.[10] Tolerance of rice varieties to deficiency of oxygen is variable and, in the same way, the grain yield. Researchers are performing investigations to understand the responses to anoxia stress by plants since, reduction of the specific amount of oxygen below optimum level, is the usual form of stress occurring during partial submergence of plant in the crop fields, stress conditions that diminishes grain yield. The behavior of the anoxia stress resistance rice variety, MAS 946-1 (Sharada) was tested and, its capacity in grain yield was superior over the Rasi variety (control) at all the 27 locations tested in the region of southern Karnataka, India.[21] Miro and Ismail[22] in a review article, analyzed the tolerance of anaerobic conditions caused by flooding during germination and early development in rice to identify the following tolerant rice varieties: Khao Hian On, Ma-Zhan (Red), Khaiyan, Kalonchi, Kharsu, and Nanhi; and based on the vegetative stage of the plants, the tolerant varieties: FR13A. FR43B, Kurkaruppan, Goda Heenati, Thavalu, IRRI 119, IRRI 154, Jalmagna, Baisbish, Rayada 16-3, Nang Dum To, and Sudu Gries.

The results obtained in the present investigation demonstrated that both germination and vigor (dry biomass weight of seedlings) of seeds of *G. hirsutum* and *G. lobatum* were diminish by the conditions of vacuum tested, facts due, in the first place, to adjusts performed in their metabolism induced by the process of adaptation to the lack of oxygen, conditions that make difficult the production of energy by the cells[23] and, in second term, to the genetic makeup of each species as it was demonstrated by Finch-Savage et al.[24] in seeds of double haploid genotypes of *Brassica oleraceae* placed to germinate under conditions of hypoxia.

Copyright: ©2019 Pérez-Mendoza et al.

In the species *G. hirsutum* and *G. lobatum* was also observed a reduction in the accumulation of dry biomass of total seedlings, when the seeds were placed to germinate under the conditions of vacuum tested. Dantas et al.[25] detected the same behavior in varieties of corn seeds, when they evaluated the germination and vigor, under conditions of hypoxia while Gazola et al.[26] found, under conditions of hypoxia, reduction in both, corn seed germination and accumulation of dry biomass of the root and the aerial section.

Regarding the effect that the condition of vacuum had and the types of substrates tested, evaluated in terms of the availability of germination, it was demonstrated that the seed in the Versa Pak substrate exhibited the highest germination, in contrast to the brown Sanita towel, in which the lowest percentage of germination was found as well as a lower accumulation of dry matter by the plantlets that emerged under the different treatments evaluated. These results may be related to the physical properties and chemical composition of each substrate, such as porosity, which is directly related to the water and oxygen supplied to the roots of the seedlings. Vence[27] mentions that the relation water-air in the substrates varies widely with the size of the particles that predominate in their composition, being one of the factors that define the size of the pores. Burés[28] also claims that a substrate with large particles and with open inner pores guarantees an adequate supply of water and high ventilation levels. Under the vacuum conditions tested, it was found that as the vacuum levels increased (0 to 700 mm Hg), the germination rates decreased, facts that could be due to the reduction of the amounts of O_2 in the media; in consequence, mitochondrial respiration, oxidation and oxygenation processes became inhibited,[29] affecting the process of germination in the species of *Gossypium* spp. It is well known that O_2 is the molecule required to produce the energy needed for germination.[30,11]These results are in concordance with those by Artola et al.[7] who evaluated the germination of seeds of *Lotus corniculatus* under vacuum conditions. It was also observed that, as O_2 levels fell down in the glass desiccators, germination of cotton seeds also decreased, probably due to the fact that the mitochondrial structures become damaged due to the conditions of hypoxia imposed, demonstrating an involvement of the activity of the enzyme superoxide dismutase, leading to the peroxidation of lipids that cause the seeds to deteriorate.[31,20] According to Batista et al.[32] this behavior could be a strategy to protect the plant, which consists in reducing the growth of the structural parts of the seedlings during their development, to save energy and maintain the minimum permissible functioning of the metabolism, particularly in the most affected regions for hypoxia. It may explain the high percentages of un-germinated seeds left, in the results obtained for the species of *G. lobatum*.

It has been easy to adjust the vacuum conditions required in this research, to obtain O_2 levels as low as 0.3 to 3%. The estimation of the amounts of oxygen left in the vacuum conditions to which the seeds were subjected in the present research, are based on the type of calculations devised by Meena et al.[33] Thus, the percentage of residual O_2 calculated for each treatment resulted as follow: at 300, 500, 600, and 700 mm Hg there are 14.57, 8.14, 4.93 and 1.71% of O_2 respectively. Meena et al.[33] calculated for 688.18 mm Hg (91.75 kPa) the value of 2.09% of O_2 and for 734.53 mm Hg (97.929 kPa), there was a remnant of 0.60% of O_2. Finally, based on the estimates of the non-linear regression models, the thresholds of the residual O_2 that could generate a negative effect on the germination of *Gossypium* spp. seeds were estimated. There were slight differences in the calculations of the germination thresholds between the two cotton species: In *G. lobatum* at 1.47% of residual O_2 (707.53 mm Hg), the germination would be reduced to zero, while in seeds of *G. hirsutum* this could occurred at 0% O_2 (765.8 mm Hg). In this regard, Heichel & Day[34] concluded that dicotyledonous species require oxygen at levels higher than 2% to activate the oxidative systems involved in seed germination.[35]

Conclusion

The vacuum vigor test chosen to determine the vigor condition of the cotton seeds, allowed their differentiation according to this criterion. The germination and vigor of the seeds in these cotton species decrease as the different levels of vacuum tested increase, since in them, the amounts oxygen also decreases in a parallel way, as it was demonstrated when calculating the amounts of oxygen present in each vacuum condition. The estimated oxygen threshold for *G. hirsutum* and *G. lobatum* determines the germination and vigor conditions of the seeds. The Versa Pak substrate promotes the greater vigor of the seeds in cotton seeds.

Acknowledgments

The first author would like to thank the CONACyT for the funds provided for her graduate studies in the Colegio de Postgraduados (COLPOS) for the facilities provided and the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) for the economic funds, as well as, for the support given for this investigation. Also, to C. José Luis Huerta Palma for the technical support given to this investigation.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Ulloa M, Abdurakhmonov IY, Pérez MC, et al. Genetic diversity and population structure of cotton (*Gossypium* spp.) of the new world assessed by SSR Markers. *Bot*. 2013;91:251–259.
2. Gil RA, López ME. Germination characteristics of native cotton, *Gossypium* spp., seeds of green, lilac and brown fiber. *Rebiol*. 2015;35:39-46.
3. ISTA. International rules for seed testing. *International Seed Testing Association*, Bassersdorf, Switzerland; 2013.
4. Hyatt JE, Tekrony DM. Factors influencing the saturated salt accelerated aging test in tomato and onion. *Seed Sci Tech*. 2008; 36:534–545.
5. McDonald MB. Assessment of seed quality. *Hort Sci*. 1980;15:784–788.
6. Filho MJ. Seed vigor testing: an overview of the past, present and future perspective. *Scient Agric*. 2015;72:363–374.
7. Artola A, Carrillo CG, García SG. A seed vigor test for *Lotus corniculatus* L. based on vacuum stress. *Seed Sci Tech*. 2004;32: 573–581.
8. Magneschi L, Perata P. Rice germination and seedling growth in the absence of oxygen. *Ann Bot*. 2009;103:181–196.
9. Ray S, Vijayan J, Sarkar R. Germination stage oxygen deficiency (GSOD): An emerging stress in the era of changing trends in climate and rice cultivation practice. *Front Plant Sci*. 2016;7:671.
10. Al-Ani A, Bruzau F, Raymond P, et al. Germination, respiration and adenylate energy change of seeds at various oxygen partial pressures. *Plant Physiol*. 1985;79:885–890.

Copyright: ©2019 Pérez-Mendoza et al.

11. Yasin M, Andreasen C. Effect of reduced oxygen concentration on the germination behavior of vegetable seeds. *Hort Environ & Biotechnol.* 2016;57:453–461.
12. Ellis MH, Millar AA, Llewellyn DJ, et al. Transgenic cotton (*Gossypium hirsutum*) over-expressing alcohol dehydrogenase shows increased ethanol fermentation but no increase in tolerance to oxygen deficiency. *Austr J Plant Physiol*. 2000;27:1041–1050.
13. Bradford KJ, Come D, Corbineau F. Quantifying the oxygen sensitivity of seed germination using a population-based threshold model. *Seed Sci Res*. 2007;17:33–43.
14. Bernardo SK, Ursulino AE, Alcântara BRL, et al. Substratos para Germinação e Vigor em Sementes de C*rataeva tapia* L. *Rev Brasil Biociênc*. 2007;5:111–113.
15. Muller KE, Benignus VA. Increasing Scientific Power with Statistical Power. *Neurotoxicol & Teratol*. 1992;14:211–219.
16. Castelloe JM. Sample Size Computations and power analysis with the SAS system. *Proceedings of the Twenty-fifth Annual SAS Users Group International Conference*. 2000;265–25.
17. Statistical analysis system institute (SAS Institute Inc). SAS guide for personal computers. version 9.00 edn. USA; 2000:1028.
18. Azcon BJ, Talon M. Fundamentals of plant physiology. *McGraw-Hill Interamericana de España*. 2008;651 .
19. Doria J. Revisión bibliográfica. generalidades sobre las semillas: su producción, conservación y almacenamiento. *Cult Trop*. 2010;31:74–85.
20. Abreu LAS, Moreira-de-Carvalho ML, Gomes PCA, et al. Deterioration of sunflower seeds during storage. *J Seed Sci*. 2013;35: 240–247.
21. Venkatesh-Gandhi R, Rudresh NS, Shivamurthy M, et al. Performance and adoption of new aerobic rice variety MAS 946-1 (Sharada) in southern Karnataka. *Karnataka J Agric Sci*. 2012;25(1):5–8.
22. Miro B, Ismail AM. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* **L.).** *Front Plant Sci*. 2013;4(269):1-18.
23. Jiménez SJC, Moreno FLP, Magnitskiy S. Plant responses to stress due to flooding. A review. *Rev Colomb Cienc Hort*. 2012;6: 96–109.
24. Finch SWE, Come D, Lynn JR, et al. Sensitivity of *Brassica oleracea* seed germination to hypoxia: a QTL analysis. *Plant Sci*. 2005;169:753–759.
25. Dantas BF, Aragão CA, Cavariani C, et al. Efeito da duração e da temperatura de alagamento na germinação e no vigor de sementes de milho. *Rev Brasil Sement*. 2000;22:88–96.
26. Gazola D, Zucareli C, Camargo MC. Comportamento germinativo de sementes de cultivares de milho sob condições de hipóxia. *Cient Jaboticab*. 2014;42:224–232.
27. Vence LB. Water-air availability in plant substrates. *Ciencia del Suelo*. 2008;26:105–114.
28. Burés PS. Substrates: physical, chemical and biological properties. 2002.
29. Koppitz H. Effects of flooding on the amino acid and carbohydrate patterns of *Phragmites australis*. *Limnologica*. 2004;34:37–47.
30. Taylor AG. Seed storage, germination and quality. In: The physiology of vegetables crops; In: Wien HC, editor. CAB International, Wallingford, UK; 1997:1–36.
31. Bailly C. Active oxygen species and antioxidants in seed biology. *Seed Sci Res*. 2004;14:93–107.
32. Batista CUN, Medri ME, Bianchini E, et al. Tolerância à inundação de *Cecropia pachystachya* (Trec. Cecropiaceae): aspectos ecofisiológicos e mor-foanatômicos. *Acta Bot Brasil*. 2008;22:91–98.
33. Meena MK, Chetti MB, Nawalagatti CM, et al. Vacuum packaging technology: a novel approach for extending the storability and quality of agricultural produce. *Adv in Plants & Agricult Res*. 2017;7: 00242.
34. Heichel GH, Day PR. Dark germination and seedling growth in monocots and dicots of different photosynthetic efficiencies in 2% and 20.9% oxygen. *Plant Physiol*. 1972;49:280–283.
35. Bewley JD, Black M. Seeds physiology of development and germination. 3rd ed, *Plenum Press*, New York, 1994;445.

Citation: Pérez-Mendoza C, Tovar-Gómez MR, Carrillo-Castañeda G, et al. Vacuum test and type of substrate on the germination and vigor of seeds of *Gossypium* spp. *Horticult Int J.* 2019;3(3):166–171. DOI: 10.15406/hij.2019.03.00125