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ISSN: 2594-0252

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Año 17 • Volumen 17 • Número 2 • febrero, 2024

Colegio de Postgraduados

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Germination test of maize (*Zea mays* L.) seeds in a NaCl solution

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ABSTRACT
Objective: To evaluate the germination behavior and to define the initial vigor of maize seeds.
Methodology: The genetic material consisted of four batches of 20 hybrid seeds each (M-2014, SR-2014, SR-2012, and SR-2011), which were germinated in a NaCl solution (0, 5, 10, and 15 dS m^{-1}) in a germination chamber. A randomized complete block experimental design was used, in which the seed batch and salinity level were the experimental units, with four repetitions each. Seeds with defined radicle and plumule were counted for evaluation.
Results: As a result of the comparison of the three batches from the same environment (production site) and different years, the statistical analysis indicated that the longest-lived seeds have a lower germination percentage. Similarly, when batches from different production environments and the same year were compared, M-2014 obtained the highest average germination percentage (92.19%). When the maximum level of salinity (15 dS m^{-1}) was evaluated, an average 41.25% loss in germination was observed.
Conclusions: The test efficiently discriminated seed batches, based on speed and uniformity in NaCl-based germination, therefore providing acceptable results for the evaluation of the viability and vigor of a seed batch.

Keywords: Hybrid, saline solution, initial seed vigor, physiological quality.

Citation: Santillán-Ángeles, A., Quevedo-Nolasco, A., Mejía-Contreras, A., & Gavi-Reyes, F.† (2024). Germination test of maize (*Zea mays* L.) seeds in a NaCl solution. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i2.2551

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: March 21, 2023.
Accepted: January 15, 2024.
Published on-line: April 05, 2024.

Agro Productividad, 17(2). February. 2024. pp: 21-26.

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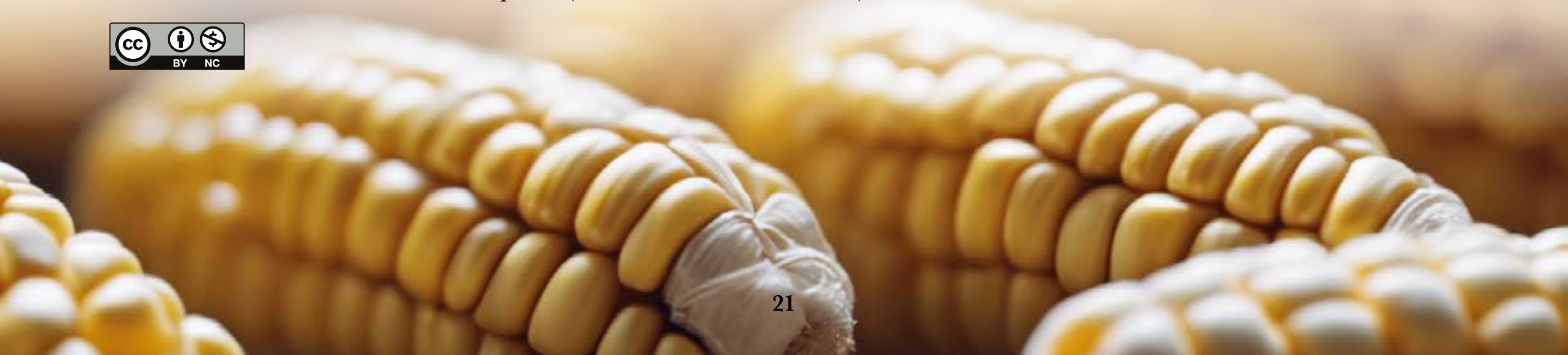
INTRODUCTION

In order to establish a plantation, around 80% of crops require seeds. In addition, their establishment must be ensured, in such a way that a vigorous and uniform emergence of the seedlings guarantees a good development and a better performance. At the same time, in order to ensure that a seed batch is adequately established in the field, their germination capacity should be determined. In this regard, several tests help to infer their physiological potential; however, the seeds are prone to deterioration for different reasons, which limit their germination. The low germination rate of a given seed batch is associated with early signs of disorganization of the cell membrane: abnormalities can occur in seedlings, when an early death of tissues in different parts of the seed (particularly in meristematic tissues) takes place (Marcos-Filho *et al.*, 2015).



In terms of their viability and vigor, the quality of the seeds is considered a physiological quality and is established during the first stage of crop development. Viability refers to the percentage of seeds that can germinate and develop into a normal plant under optimal conditions. Nevertheless, the full germination of the seed is insufficient for agricultural purposes; In addition, it must be able to germinate in adverse field conditions; this characteristic is known as vigor (Bradford, 2004). Therefore, many researchers consider that the analysis of the physiological quality of a seed batch must include their viability, high germination capacity, and vigor, if they are to establish and produce new individuals.

A wide variety of standardized tests are used to determine the physiological quality of a seed batch, but the standard germination test is the most used. Another way to determine physiological quality is the application of the accelerated aging test, which is used to determine vigor. However, this test may have limitations —*e.g.*, a high moisture content can accelerate the deterioration process of the sample (Ramos *et al.*, 2017).

In this regard, Jianhua and McDonald (1996) proposed the use of saline solutions in seed testing conditions, in order to reduce water absorption and improve the uniformity of the results through the reduction of the incidence of fungi in the samples. In its turn, the use of saline solutions to evaluate seed batches helps to determine the germination capacity and to produce a seedling under stress conditions. In addition, it is indicative of excellent genetic potential, at least at this stage (Bernstein and Ayers, 1953; Pearson *et al.*, 1966). However, Grieve and Suárez (1997), Katembe *et al.* (1998), and Khan and Ungar (1998) point out that a saline medium could inhibit germination and seedling growth, as a consequence of low osmotic potential and ionic toxicity. Specifically, Allen *et al.* (1995) mention that NaCl affects the permeability of plasma membranes and increases the influx of external ions and the efflux of systolic solutes in plant cells.

A successful germination will depend on the transportation of water to the tissues that surround the embryo and on the relationship of the water potential between the seed and the medium. A potential gradient prevents the absorption of water by the seeds; therefore, when the osmotic potential of the solution is more negative than the potential of the embryo cells, germination occurs (Aparecida *et al.*, 2015; Cavalcante and Pérez, 1995; Carvalho and Nakagawa, 2000).

The objective of this work was to evaluate the germination behavior of maize seeds in a saline medium, using a NaCl solution to define the initial vigor.

MATERIALS AND METHODS

This research was carried out in the seed analysis laboratory of the Colegio de Postgraduados-Servicio Nacional de Inspección y Certificación de Semillas, located in Montecillo, Estado de Mexico. The genetic material consisted of four batches of hybrid maize seeds, which were produced in different environments-years (M-2014, SR-2014, SR-2012, and SR-2011).

The test was carried out in a germination chamber, with a controlled environment of 25 °C and 100% relative humidity. Twenty seeds from each batch were placed in Petri dishes, whose base was lined with a "medium pore" filter paper. A NaCl saline solution was used at four levels of electrical conductivity (EC): 0, 5, 10, and 15 dS m^{-1}. To that effect, Maas and Hoffman (1977) pointed out that 100% of the production of the maize crop is affected by an EC of 12 dS m^{-1}. This solution was applied periodically to constantly maintain a ≤2mm sheet and activate the imbibition process.

The observation of the seeds began from day one; the germination was considered to have taken place when the radicle and plumule were defined. The counting started on day 4.

A randomized complete block experimental design with four repetitions was used. The analysis of variance and comparison of means were carried out with the LSD (Fisher's least significant difference) test, with an error probability of $\alpha = 0.05$, using the SAS OnDemand for Academics (Statistical Analysis System) free online access statistical software.

RESULTS AND DISCUSSION

The result of the experiment showed significant differences among batches (environment-year of production) and salinity level. The interaction between these two factors is not significant (Table 1).

The comparison of means between the batches (environment-year of production) and salinity level (Table 2) shows that the technique (germination test in saline solution) makes a statistically significant contribution to determine the germination of maize seed batches. A comparison of means test was carried out to evaluate the differences of the NaCl solution between batches and salinity level.

In this regard, when the seed is subjected to different salinity levels, its behavior indicates a tendency towards a decrease in the germination percentage, as the concentration of the saline solution increases. When the batches from two different environments and the same production year are compared (M-2014 and SR-2014), the former has the highest average germination percentage (92.19%), which shows that the production environment influences the final quality of a given seed batch. In this regard, McDonald (1999) points out that seed deterioration is different in each species and genotype, as a result of environmental and biological factors; furthermore, this phenomeon is not uniform in every seed or in every batch. Therefore, the environment influences the

Table 1. Analysis of variance of the germination behavior in saline solution of maize seeds produced in different environment-years.

Variation factor	Degrees of freedom	Medium square	(Pr≥F)
Environment-year of production	3	4,304.17	<0.0001
Salinity level	3	1,122.92	<0.0001
Environment-year of production * Salinity level	9	69.44	0.5778
Error	48	82.03	
R^2	0.81		
Variation coefficient (VC %)	12.99		
Average	69.68		

Table 2. Tukey's test between the batch (environment-year of production) and salinity level factors in the germination of maize seeds.

Environment-year of production	**Salinity level (NaCl, dS m^{-1})**				**Germination average (%)**
	0	**5**	**10**	**15**	
M-2014	96.25	96.25	88.75	87.50	92.19 a
SR-2014	76.25	75.00	73.75	61.25	71.56 b
SR-2012	67.50	68.75	53.75	41.25	57.81 c
SR-2011	65.00	65.00	53.75	45.00	57.18 c
Germination average (%)	76.25	76.25	67.50	58.75	
	a	a	b	c	

α: 0.05

development of seeds, particularly when the production environment is different from the environment in which they are sown: the place where the seeds are produced can cause major modifications in their chemical composition (Carvalho, 1979; Mayer and Poljakoff-Mayber, 1982).

Similarly, when the three batches from the same environment and different year are compared (SR-2014, SR-2012, SR-2011), the statistical analysis indicates that longest-lived seeds have a lower germination percentage. These results demonstrate that the aging of a seed batch is reflected in the loss of quality, as mentioned by Marcos-Filho and McDonald (1988): seed aging is a sequential set of biochemical and physiological events that progressively reduce quality, leading to the loss of viability, affecting potential returns, and increasing economic losses. In his turn, Carvalho (1979) mentions that seeds can lose their viability as a result of the mechanical damage that poor handling (post-harvest and storage) could cause on a seed batch.

When the effect of salinity levels is compared, a marked decrease in the germination percentage is observed (at a higher level of salinity). In this sense, the results match the findings of Zahedifar (2013) who experimented with different levels of salinity and reported that the average germination rate of maize seeds decreased significantly as salinity increased. The results of Ramezani and Fatemi-Nik (2013) also match the findings of this study: they reported that an increased concentration of NaCl reduces the germination rate, as a consequence of the increase in osmotic pressure and the reduction of water absorption by maize seeds. Therefore, a delay in water absorption by the seeds is caused by the toxic effects of ions which reduce the permeability of the plasma membrane of the embryo (Laynez *et al.*, 2008).

Zahadifar (2013) recorded a 95% decrease in germination when a 2% concentration of NaCl is applied. In the present experiment, a 41.25% loss in the average germination percentage was observed during the evaluation of the maximum salinity level of 15 dS m^{-1} (1.31% concentration). Therefore, in comparison with the ISTA (2004) standard germination test, this test was also an acceptable tool to evaluate the viability and vigor of a seed batch, because it can efficiently discriminate seed batches based on their germination speed and uniformity.

CONCLUSIONS

In conclusion, the vigor of maize seed differs based on the environment-year of production and its response to the level of salinity: a salinity level of 15 dS m^{-1} enables the representative qualification of the physiological quality of a seed batch. Therefore, the germination test used to define the initial vigor of a seed batch resulting from the application of a saline solution is a quick, effective, and reliable way to quantify the physiological quality of maize seeds.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of the late Mr. Francisco Gavi Reyes (ScD) to this study.

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