

Incidence of water stress induced by Polyethylene glycol 6000 on the germination process of a hybrid and a cultivar of maize (*Zea mays* L.)

Incidencia del estrés hídrico inducido por Polietilenglicol 6000 en el proceso de germinación de un híbrido y un cultivar de maíz (*Zea mays* L.)

Incidência do estresse hídrico induzido pelo polietilenoglicol 6000 no processo de germinação de um híbrido e uma cultivar de milho (*Zea mays* L.)

Páez López, A. ¹; Rojas Calzada, D. ²; Hernández Pérez, T. ²; Maqueira López, L. A. ^{1*}, Blanco Hernández, A. de la C.¹; Tavares Cruz, A. ¹; Vázquez Alarcón L. M.¹

1. Unidad científica Tecnológica de Bases Los Palacios (UCTB Los Palacios), Instituto Nacional de Ciencias Agrícolas (INCA), Carretera Sierra Maestra, km 1½, Los Palacios, Pinar del Río, Cuba.
2. Universidad de Pinar del Río (UPR), Calle Martí, No. 270 final, Pinar del Río, Cuba.

* Corresponding author: maqueiralopez@gmail.com

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Abstract

The research was conducted in the Ecophysiology Laboratory of the Los Palacios Basic Scientific and Technological Unit (UCTB-LP), belonging to the National Institute of Agricultural Sciences, Cuba, with the objective of evaluating the impact of water stress induced by different concentrations of polyethylene glycol 6000 on the germination process of two maize cultivars, Hybrid and Criollo. A completely randomized experimental design was used, with five treatments and three replicates. Each group of seeds (50 seeds) was placed at the bottom of each glass Petri dish on a layer of filter paper moistened with different concentrations of polyethylene glycol-6000 (20, 25, 30, 35%), and a control with distilled water. Germination dynamics, germination percentage, and the Maguire index were determined. The 95% confidence interval for the probability of error was determined for the data of each variable using the SPSS 20.0 statistical package for Windows. The indicators Germination Dynamics, Germination Percentage, and Maguire Index are suitable for evaluating the impact of water stress induced by Polyethylene Glycol 6000 on maize seed germination. Hybrid maize exhibits greater germination capacity under optimal conditions, but its vigor is more significantly affected by severe water deficit compared to native varieties.

Keywords: Maguire Index, Tolerance, Drought

Resumen

La investigación se realizó en el laboratorio de Ecofisiología de la Unidad Científico-Tecnológica de Base Los Palacios (UCTB-LP), perteneciente al Instituto Nacional de Ciencias Agrícolas, Cuba, con el objetivo de evaluar la incidencia del estrés hídrico inducido por diferentes concentraciones de Polietilenglicol 6000 sobre el proceso de germinación de dos cultivares de maíz, Híbrido y Criollo. Se utilizó un diseño experimental completamente aleatorizado, con cinco tratamientos y tres repeticiones, cada grupo de semillas (50 semillas) fueron ubicadas en el fondo de cada placa Petri, de vidrio, sobre una capa de papel de filtro humedecido con diferentes concentraciones de polietilenglicol-6000 (20, 25, 30, 35 %) y un testigo con agua destilada. Se determinó la dinámica de germinación, el porcentaje de germinación y el índice Maguire. A los datos de cada variable se le determinó el intervalo de confianza al 95 % de la probabilidad de error, utilizando el paquete estadístico SPSS 20.0 sobre Window. Los indicadores Dinámica de germinación, Porcentaje de germinación e Índice Maguire resultan adecuados para evaluar la incidencia del estrés hídrico inducido por el Polietilenglicol 6000 sobre la germinación de semillas de maíz. El maíz híbrido posee una mayor capacidad germinativa en condiciones óptimas, pero su vigor se afecta de manera más significativa ante condiciones de déficit hídrico severo en comparación con el criollo.

Palabras clave: Índice de Maguire, Tolerancia, Sequía

Resumo

A pesquisa foi conduzida no Laboratório de Ecofisiologia da Unidade Básica Científica e Tecnológica Los Palacios (UCTB-LP), pertencente ao Instituto Nacional de Ciências Agrícolas de Cuba, com o objetivo de avaliar o impacto do estresse hídrico induzido por diferentes concentrações de polietilenoglicol 6000 no processo de germinação de duas cultivares de milho, Híbrida e Crioula. Utilizou-se um delineamento experimental inteiramente casualizado, com cinco tratamentos e três repetições. Cada grupo de sementes (50 sementes) foi colocado no fundo de cada placa de Petri de vidro sobre uma camada de papel filtro umedecida com diferentes concentrações de polietilenoglicol-6000 (20, 25, 30, 35%), e um controle com água destilada. Foram determinados a dinâmica de germinação, a porcentagem de germinação e o índice de Maguire. O intervalo de confiança de 95% para a probabilidade de erro foi determinado para os dados de cada variável utilizando o pacote estatístico SPSS 20.0 para Windows. Os indicadores Dinâmica de Germinação, Percentagem de Germinação e Índice de Maguire são adequados para avaliar o impacto do estresse hídrico induzido pelo Polietilenoglicol 6000 na germinação de sementes de milho. O milho híbrido apresenta maior capacidade de germinação em condições ótimas, mas seu vigor é mais significativamente afetado por déficit hídrico severo em comparação com as variedades nativas.

Palavras-chave: Índice de Maguire, Tolerância, Seca

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Introduction

Corn (*Zea mays* L.), native to Mexico, has been one of humanity's staple foods for generations, especially in the Americas. Today, it is produced in 125 countries and ranks among the top three most widely cultivated crops, 75 of them, for both human and animal consumption, as it is rich in carbohydrates, fiber, and essential minerals that provide energy and promote health. It is also highly effective at naturally sequestering carbon, making it a strategic crop for food sovereignty and security in its various forms of use (Márquez, 2022; Gómez, 2023).

Global production of this crop exceeds that of any other cereal, with 850 million metric tons of grain grown on an area of 162 million hectares, for an average yield of 5.2 tha⁻¹. The United States and China are considered the largest producers, accounting for 37% and 21%, respectively, of the world total. In this regard, new records are on the horizon with relatively optimistic prospects, with projected increases of 192.9 million metric tons (FAO, 2025).

In Cuba, domestic production relies primarily on cultivars of phylogenetic origin derived mainly from Caribbean varieties, which are well adapted to the country's tropical conditions and characterized by high tolerance to pests. Despite this, their contribution to dry corn production is low, averaging 1.93 t ha⁻¹, due to water deficit, an abiotic factor that affects the cultivar's growth and yield (Téllez et al., 2021).

Some authors have reported that one of the factors that can hinder the normal development of maize and its subsequent yield is drought, either during or immediately after germination. This is the most critical stage in seedling establishment and can determine the success of crop production as the plant progresses through its various phenological stages (Channaoui et al., 2017).

Polyethylene glycol (PEG), on the other hand, is a neutral polymer available in a range of molecular weights and highly soluble in water; due to these properties, it is used to impose water stress on plants, reducing the water potential of the root zone and, consequently, the plant's water potential (Kylyshbayeva et al., 2024). The limited availability of water, due to climate change, constitutes a limiting factor for crop production; therefore, it is necessary to investigate the response of the germination process of maize cultivars to water stress induced by polyethylene glycol-6000, with results that contribute to the crop's tolerance under drought conditions. Taking the above into account, this study was conducted to evaluate the impact of water stress induced by polyethylene glycol 6000 on the germination process of a native maize cultivar and a hybrid.

Materials and Methods

The research was conducted at the Ecophysiology Laboratory of the Los Palacios Basic Scientific and Technological Unit (UCTB-LP), part of the National Institute of Agricultural Sciences; located on the southern plain of Pinar del Río Province, at 22°44' north latitude and 83°45' west longitude, at an elevation of 60 meters above sea level, with an approximate slope of 1%, according to the Atlas of Cuba.

Seeds from the Criollo maize cultivar and a hybrid were used; their main characteristics are shown in Table 1 and were obtained from the INCA germplasm bank. Using a completely randomized experimental design, 50 seeds were placed at the bottom of each glass Petri dish - 140 mm in diameter and 20 mm high-on a layer of filter paper moistened with different concentrations of polyethylene glycol-6000 (20, 25, 30, 35%), and a control with distilled water, which constituted the five treatments and three replicates, under ambient conditions.

Table 1. Main characteristics of the Criollo corn cultivar and the hybrid used in the study.

Characteristics	Hybrid	Landrace (Creole)
Plant height (cm)	176	300
Number of leaves	13–14	16–18
Grain color	Orange	Yellow-Orange
Weight of 100 grains (g)	40	43
Number of grains per ear	290	300
Ear weight (g)	162	180
Yield (t ha ⁻¹)	4.33	4.05

Germination assessments began after 48 hours and continued for 10 consecutive days; during this period, the emergence of radicles 2 mm or longer was counted daily until the process stabilized. The data obtained were used to determine the germination dynamics and percentage, as well as the Maguire index. The following formula was used to calculate the germination percentage (Eq. 1).

$$GP(\%) = \frac{\text{Number of germinated seeds}}{\text{Number of seeds sown}} * 100 \text{ (Eq. 1)}$$

The Maguire Index represents the germination rate, calculated as a time-weighted, cumulative germination time (in days) (Eq. 2), where is the percentage of seedlings that germinate during the time interval (t).

$$IM = \sum_{t=1}^n \left(\frac{G_t}{t} \right) \text{ (Eq. 2)}$$

The data for each evaluated variable was tested for normality and homogeneity of variance using Bartlett's test and the Kolmogorov-Smirnov test, respectively. Dose-response curves were plotted for germination dynamics, while the germination percentage and Maguire index were subjected to an analysis of variance with a 95% confidence level. Means were compared using Tukey's Multiple Range Test ($P > 0.05$), and the analysis was performed using Stat Graphics Plus 5.0 Centurión software running on Windows, version XV.

Results and Discussion

The germination dynamics of the native corn cultivar and the hybrid (Figure 1), under different osmotic potential conditions induced by polyethylene glycol 6000 (PEG), compared to the control (water), showed curves with variations in their maximum amplitudes but with a stable pattern across all treatments: a rapid increase in values that peaked on the third and second days for the native cultivar and the hybrid, respectively, as well as a gradual decline by the sixth day.

As expected, the control group performed best, with a peak of 20 seeds germinating on the third day and 26 on the second day for the native cultivar and the hybrid, respectively, indicating that optimal water availability allows

for faster and more synchronized germination. Meanwhile, as the concentration of polyethylene glycol 6000 - represented by the remaining treatments - increases, a proportional decrease is observed in the number of seeds that successfully germinate, because PEG 6000 creates osmotic stress. In this regard, the literature reports that osmotic stress induced by polyethylene glycol (PEG) reduces germination.

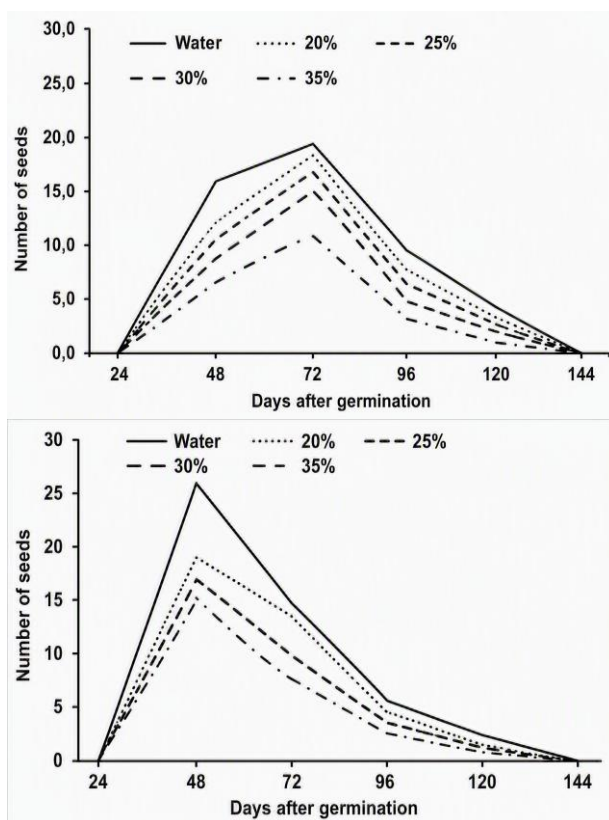


Figure 1. Germination dynamics of the native corn cultivar (top) and the hybrid (bottom) under different osmotic potential conditions induced by polyethylene glycol 6000 and a water control.

Several studies have reported that, under water stress conditions simulated with PEG-6000, there is an inverse correlation between the solution concentration and the germination percentage, with a significant reduction in radicle emergence and

seedling vigor observed as the osmotic potential becomes more negative. This effect is because PEG reduces the water potential of the medium, limiting the imbibition necessary to restart embryo metabolism, which delays or inhibits germination, especially in sensitive genotypes (Preethi et al., 2025).

Meanwhile, the decline observed after the third day indicates the completion of the germination process, while the similar trend of the curves suggests that, although osmotic stress delays or limits the total number of seeds that germinate, it does not drastically alter the rate of the process. The treatment with 35% polyethylene glycol 6000, which falls below the others, represents the most severe level of water stress, as evidenced by a marked inhibition of the seeds' biological potential, resulting from the mobilization of nutrient reserves and the compromise of the energy supply necessary for the germination process.

Several studies have been conducted by Verma et al., 2023; Sousa et al., 2023; and Husain et al., 2025 on the response of plants grown under water stress during early stages, with the aim of understanding the mechanisms of adaptation to this water deficit condition, where germination dynamics and germination rates were found to be highly dependent on water stress conditions, since water availability is essential for activating the metabolic processes that lead to the emergence of the radicle, marking the onset of germination.

In general, the hybrid corn exhibited an earlier and more pronounced peak in germination, with the highest activity occurring on the second day, indicating greater uniformity and

initial speed. In contrast, the native cultivar showed a more gradual response, peaking on the third day in most treatments - a pattern suggesting an adaptive strategy to variable environmental conditions. Under water stress induced by PEG 6000, the hybrid experienced a more drastic reduction in the number of germinated seeds compared to its control, while the native cultivar maintained a more stable temporal dynamic, with fewer fluctuations relative to its own control - a trait typical of resilient local varieties (Preethi et al., 2025).

In this regard, Pérez-Hernández (2018) suggests that low concentrations of PEG-6000 may induce the expression of anti-stress genes, as well as numerous enzymes that accelerate metabolic reactions during the germination stage. Similar results were reported by Laynez et al. (2007), who observed differences among cultivars of the same species, a phenomenon attributed to variations in internal osmotic forces during water uptake.

Choi et al., 2024, detail how osmotic stress in rice delays germination time and reduces α -amylase activity and soluble sugar content at all stages of germination, which directly affects the size of the radicle and coleoptile. In maize, it has been observed that water stress induces changes in lipid peroxidation and the activity of enzymes such as catalase and aminotransferases, indicating an adaptive response at the biochemical level during the early stages, although growth is severely reduced (Kolesnikov et al. 2025). Therefore, these studies collectively confirm that the

dynamics and success of germination under water deficit depend critically on water availability, as water is the essential trigger for the metabolic processes leading to radicle emergence (Choi et al., 2024)

The germination rate for the control treatment of both the native cultivar and the hybrid was over 90% (Figure 2), demonstrating that, under optimal conditions without water stress, their seeds can exhibit high germination potential.

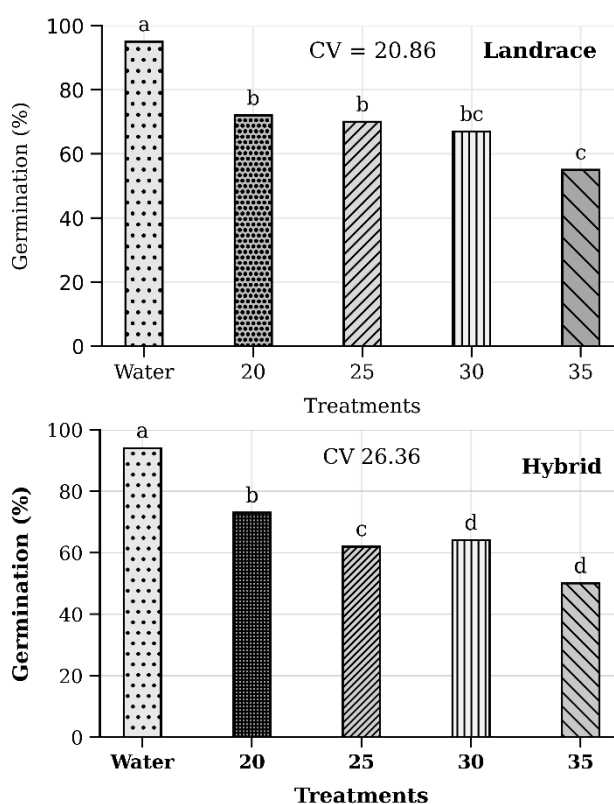


Figure 2. The percentage of the native corn cultivar and the hybrid under different osmotic potential concentrations induced by polyethylene glycol 6000 and water control.

As the concentration of polyethylene glycol in the treatment's increases (20, 25, 30, and 35%), a decreasing trend in this variable is observed, which is consistent with the action of PEG. The decline is significant starting from the

first applied stress level, dropping from over 90% in control to approximately 72% in the treatment with 20% polyethylene glycol concentration, for both the native cultivar and the hybrid.

For the native cultivar, a certain degree of stability was observed in the treatments with concentrations between 20% and 25%, with the germination rate dropping only to 70%, while for the hybrid, the rate continued to decline, reaching values of around 60% at 25% and 30% polyethylene glycol concentrations. However, the most pronounced impact occurred at the highest concentration, with a germination rate below 60% for the native cultivar and 50% for the hybrid.

In this regard, it is noted that the germination rate is the most important indicator for evaluating seed production, since this value is used for the certification and marketing of the product as a benchmark for quality; furthermore, it must be taken into account when determining the seeding rate to be used under field conditions and the agronomic management practices that ensure uniform irrigation to guarantee a high germination rate. The optimal value is 80% or higher; although a rate as low as 60% can be used, the quantity of seeds must be increased to compensate for the low germination rate. When the germination rate is below 60%, the seed is not considered suitable for planting, as was the case with the results obtained at a concentration of 35% in Treatment 5 (Gallardo, 2024).

When comparing the responses of both cultivars to increasing concentrations of PEG (from 20% to 35%), the native corn showed a more gradual and consistent decline in its germination rate, whereas the hybrid corn's response was slightly more erratic, with a sharp drop in the 25% concentration treatment, followed by a slight recovery in the 30% treatment, and finally reaching its lowest point in the 35% treatment. This difference suggests that, although both are sensitive to osmotic stress, the mechanisms of tolerance to water availability differ, with the native cultivar performing better.

Seeds store reserve substances that serve to nourish the new seedling once root emergence occurs. For these reserve substances to become part of the metabolic pathways of the embryonic axis or the embryo, they must first undergo hydrolysis so they can be transported to these growing organs via the appropriate pathways; this is why water treatment yields a better response in terms of germination percentage for both cultivars.

Therefore, under drought conditions, both the native cultivar and the hybrid experience a reduction in their initial establishment, which indicates the need to consider water stress tolerance when selecting cultivars for areas prone to low water availability, since water potential is reduced, limiting the imbibition process and the availability of water for the seeds - a fundamental aspect for the hydration of the enzymes and substrates involved in the various biochemical reactions that, in turn, initiate the germination process (Pérez-Hernández, 2018).

The Maguire Index (MI) obtained (Figure 3) for the control treatment of native corn seeds was approximately 17-18 units, higher than that of the treatments with different concentrations of polyethylene glycol, which confirms that the absence of osmotic stress allows for a faster and more uniform germination process, since the seeds are under conditions suitable for germination.

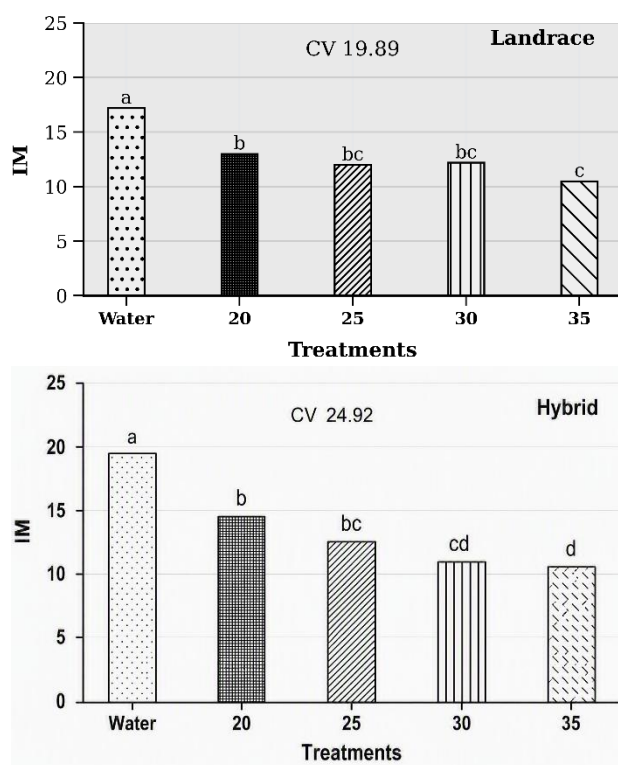


Figure 3. Maguire index of seeds from the native corn cultivar and the hybrid under different osmotic potential conditions induced by polyethylene glycol 6000 and water control.

Studies conducted on soybeans (Díaz et al., 2019) reported a reduction in the germination rate of seeds subjected to simulated drought, which the authors attribute to a lack of energy for the initiation of germination, since this energy is obtained from increases in the rate of

respiration following imbibition, which occurs more slowly in the presence of low osmotic potentials.

When PEG was added at concentrations of 20, 25, 30, and 35%, a decrease in this index was observed, reflecting the impact of water stress on the seed's imbibition capacity and the activation of the metabolic processes necessary for germination. As the concentration of the osmotic agent increases, water availability to the embryo decreases, leading to slower and less uniform germination, as demonstrated by the decrease in the bars corresponding to the treatments studied. Furthermore, relative stabilization is evident among the 20%, 25%, and 30% treatments, where the Maguire index remains within a similar range, slightly above 12. However, the 35% treatment shows the most marked decrease, reaching the lowest value of all treatments (close to 10). This behavior suggests that there is a water tolerance threshold in the Criollo cultivar, beyond which osmotic stress significantly impairs germination rate, drastically limiting seedling vigor as water availability decreases.

On the other hand, the hybrid shows a decreasing trend as osmotic stress increases. Under control conditions, it exhibits the highest value, close to 20, reflecting optimal germination speed and uniformity. This response is due to the absence of water constraints, which allows metabolic processes to be efficiently activated and maximum germination potential to be achieved under the evaluated conditions. As PEG levels increase, the index decreases, since water limitation directly affects germination, delaying radicle emergence and reducing the rate of

germinated seeds per unit of time. However, in the 30% and 35% treatments, the index tends to stabilize at a value close to 11, suggesting that, beyond a certain osmotic stress threshold, the germination process reaches a baseline state where water limitation is so severe that the germination rate no longer varies drastically, possibly reaching a response limit for this hybrid under the evaluated conditions.

In the control treatment, hybrid corn exhibits an index that exceeds that of the native variety, but with a more pronounced and consistent decline as the stress level increases, suggesting that, although the hybrid has greater germination capacity under optimal conditions, its vigor may be more significantly affected under conditions of severe water deficit compared to the native variety.

On the other hand, the Criollo corn shows relative stabilization in its Maguire Index, with very similar values among the 20%, 25%, and 30% treatments, followed by a subsequent decline in the treatment with a 35% polyethylene glycol concentration, which could indicate greater resilience or adaptive tolerance of the Criollo cultivar to moderate levels of osmotic stress. In this regard, the data suggests that, although the hybrid grows faster under favorable conditions, native corn exhibits a less variable response to increased water stress, maintaining a constant germination capacity across the different treatment levels.

The Maguire Index is a variable that weights cumulative germination over time; several authors highlight its importance for analyzing germination behavior under various experimental conditions, as it combines the parameters of germination capacity, rate, and uniformity to assess germination quality, the higher the result, the higher the seed quality and the better the response of the cultivars to conditions during the germination process (Romero et al., 2017). This index has been used in the literature for the early selection of genotypes tolerant to stress conditions (Da Vitória et al., 2018) and in studies to identify the effect of temperature on the germination process of bean seeds (Maqueira et al., 2021).

Similar behavior has been observed in corn seeds (Tsougkrianis et al., 2009), native corn and beans (Aguilar-Benítez et al., 2014), and basil (Ojeda-Silvera et al., 2013), which offers the possibility of identifying materials that respond appropriately under water stress conditions.

The germination response and recovery of growth after or during water stress will depend on the type of material being used. The more negative the potential, the more germination is reduced (Ojeda-Silvera et al., 2013); the use of polyethylene glycol retains water in the seed's environment, preventing the hydration of structures essential for the activation of recovery systems and the mobilization of reserves, which delays the germination process; however, it has been used as an osmotic conditioning technique to improve germination in terms of uniformity and speed (Marín et al., 2007).

The effects of water stress during germination range from low germination rates and poor seedling development to the inhibition of germination (Zheng et al., 2016), because seeds do not obtain the minimum amount of water required to initiate the process. In the case of maize, it is known that 300 grams of water per kilogram of whole seed weight and 500 grams per kilogram of embryo weight are required for the seed to lose its tolerance to desiccation, thereby making the germination process irreversible (Rangel-Fajardo et al., 2014).

Conclusions

The indicators "Germination Dynamics," "Germination Percentage," and "Maguire Index" are suitable for evaluating the effect of water stress induced by polyethylene glycol 6000 on the germination of corn seeds. Hybrid corn has a higher germination capacity under optimal conditions, but its vigor is more significantly affected under conditions of severe water deficit compared to native corn.

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