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Original article

Spirulina extracts reverse the effects of simulated water deficit with PEG 6 000 in corn

Extractos de espirulina revierten los efectos del déficit hídrico simulado con PEG 6 000 en maíz

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ABSTRACT: The water deficit delays the germination and initial growth of maize seedlings and the application of biostimulants can reverse this effect. The objective of this research was to determine the effects of two ethanolic extracts of spirulina (*Arthrospira platensis*) on the germination and initial growth of maize seedlings subjected to simulated water deficit with PEG 6 000. Two experiments were performed using cultivar P-7928. At the first one, desinfected seeds were placed to germinate, during seven days, in water, PEG 6 000 15 % and PEG 6 000 15 % supplemented with different concentrations (1, 0.5 and 0.1 µL mL^{-1}) of two ethanolic extracts of spirulina (SpE 1 y SpE 2). At the end of experiment, final percentage of germination and some indicators of seedling growth were evaluated. In the second one, the best performing spirulina extract was used with the same concentrations proceeded in a similar way to the first experiment. The germination dynamics was followed every 12 hours and final percentage of germination (% G), germination velocity (GV), mean germination time (MGT), germination rate (GR) were calculated. Results showed that the imposed osmotic stress delayed germination but did not affect the final percentage and decreased significantly the shoot length. The addition of an ethanolic extract of spirulina (SpE 2) at 1.0 µL mL^{-1} concentration completely reversed the delay in germination imposed by PEG 6000 and generally stimulated the seedling growth.

Key words: osmotic stress, germination, *Spirulina platensis*, *Zea mays*.

RESUMEN: El déficit hídrico retrasa la germinación y el crecimiento inicial de plántulas de maíz y la aplicación de bioestimulantes puede revertir este efecto. El objetivo de esta investigación fue determinar los efectos de dos extractos etanólicos de espirulina (*Arthrospira platensis*, originalmente incluidas en el género *Spirulina*) en la germinación y el crecimiento inicial de plántulas de maíz sometidas a déficit hídrico simulado con PEG 6000. Se ejecutaron dos experimentos utilizando el cultivar de maíz P-7928. En el primero, las semillas desinfectadas se pusieron a germinar durante siete días en agua, PEG 6 000 15 % y PEG 6 000 15 % suplementado con diferentes concentraciones (1, 0,5 y 0,1 µL mL^{-1}) de dos extractos etanólicos de espirulina (ESp1 y ESp2). Al final del experimento, se evaluaron el porcentaje final de germinación y algunos indicadores del crecimiento de las plántulas. En el segundo, se utilizó el extracto de espirulina de mejor comportamiento con las mismas concentraciones y se procedió de forma similar al primer experimento. Se siguió la dinámica de germinación cada 12 horas y se calcularon el porcentaje final de germinación (% G), la velocidad de germinación (VG), el tiempo medio de germinación (TMG) y la tasa de germinación (TG). Los resultados mostraron que el estrés osmótico impuesto retrasó la germinación, pero no afectó el porcentaje final y disminuyó, significativamente, la longitud del vástago. La adición de 1,0 µL mL^{-1} del extracto etanólico de espirulina (ESp 2) revirtió totalmente el retraso de la germinación impuesto por el PEG 6 000 y estimuló, de forma general, el crecimiento de las plántulas.

Palabras clave: estrés osmótico, germinación, *Spirulina platensis*, *Zea mays*.

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INTRODUCTION

Corn (*Zea mays* L.) is one of the three most important cereals in the world, together with wheat and rice. Currently, it is produced in 125 countries, approximately 201 million hectares (ha) cultivated and its production reaches 1224.5 million tons (t), which means an increase of 6.4 % compared to the previous year. The main producers account for 65.2 % of world corn production: the United States with 30.3 %, China 24.1 % and Brazil 10.9 %, respectively (1).

Corn has been grown in Cuba since the time of the aborigines and is a staple food for human, livestock and poultry nutrition (2). In Cuba, 126 000 ha are dedicated to this crop, with an average yield of 1.89 t ha^{-1} (3).

One of the key stages in the development of a crop is the successful establishment of seedlings; therefore, the lack of moisture during or immediately after planting affects germination and initial growth, reducing plant density and final yield (4, 5). This effect is especially marked in maize, where rapid seedling dehydration is associated with poorly developed root systems (6). In this crop, it has been estimated that drought, during germination, can cause losses in the number of plants, which causes yield losses comparable to those produced by drought during the flowering stage (7).

Regarding the effect of water deficit on the germination process of corn seeds, when comparing drought tolerant and non-tolerant genotypes, it was found that the imbibition phase is the one that is delayed, due to the fact that the osmosis process is delayed when the water deficit increases, being more marked this delay in the non-tolerant genotypes (8). The first process affected by water deficit is growth, which begins with seed germination; this includes the phases of imbibition, metabolism and the growth beginning of the structures found in the embryo (4).

In laboratory studies, soil water deficit can be simulated by using solutions with defined water potentials. Several studies have been conducted to evaluate the behavior of different species under simulated water deficiency with polyethylene glycol (PEG) (9-11). This compound has the advantage that, in addition to increasing the osmotic potential of the solution, it is metabolically inactive.

On the other hand, spirulina (*Arthrospira platensis*) is a type of cyanobacterium that is of great interest due to its high nutritional values (12, 13). It is widely used in pharmaceuticals and cosmetics, although it is little exploited in agriculture. Due to its chemical composition rich in proteins, essential polyunsaturated fatty acids and vitamins, as well as xanthines, phycobiliproteins, carbohydrates, minerals, phytohormones, antioxidants and polysaccharides, it is an excellent biological complement (12, 14).

The effect of *Arthrospira platensis* application on corn has been studied, where it increased germination and growth (15). Also, in recent years, its potential to protect plants against abiotic stress has been investigated. For example, the use of aqueous extracts of *Spirulina* spp. improved the tolerance of wheat to salinity, increasing the content of proteins, carbohydrates, total phenols and antioxidants in seedlings (16). However, the effect of spirulina extracts on plants subjected to default water stress has been little studied. For this reason, the objective of this work was to evaluate the effect of two spirulina extracts on germination and initial growth of maize seedlings cv. P-7928, subjected to simulated water deficit with PEG 6 000.

MATERIALES AND METHODS

Experiments were carried out at the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas municipality, Mayabeque province.

Preparation of spirulina alcoholic extracts

The alcoholic extracts of spirulina were prepared as follows: for extract number 1 (ESp1): 10 g of spirulina powder were taken from the Basic Business Unit (UEB) Espirulina, of Jaruco, and dissolved in 100 mL of 90 % ethanol. This homogenate is shaken for 10 minutes every 24 hours for 21 days, after which time it is filtered and stored at 4 ºC. For extract number 2 (ESp2) this methodology was followed, but the dissolution was carried out in 100 mL of 70 % ethanol for 10 days.

Effect of different concentrations of alcoholic extracts of spirulina on the germination of corn seeds cv. P-7928, under water deficit conditions

Polyethylene glycol (PEG-6000) (Sigma-Aldrich) 15 % (-0.295 MPa) solutions prepared with sterile distilled water were used to simulate water stress. The experiment was repeated twice.

Seeds of maize (*Zea mays* L.) cv. P-7928, which were disinfected with NaClO 5 % for 10 minutes. After being washed three times with sterile distilled water, they were placed in sterilized Petri dishes (15 seeds per dish and five dishes per treatment), to which 15 mL of each of the test solutions were added (Table 1).

Plates were placed in the dark in a growth chamber at 28-30 ºC for seven days. After this time, the final germination percentage was evaluated, and 25 seedlings per treatment were selected to evaluate the length of the stem and radicle and the dry mass of the seedlings.

In the second experiment, the ethanolic extract with the best response with the three concentrations was used and the procedure was similar to the first experiment, except that Hoagland's nutrient solution was used for the preparation of the test solutions. In this case, the germination dynamics was followed by counting the germinated seeds every 12 h (12, 24, 36, 48, 60, 60, 72, 84, 96, 108, 120, 132, 144 hours) for six days and expressed as a percentage. In addition, the performance of different germination indicators was calculated according to the methodology proposed by the International Seed Testing Association (ISTA):

Table 1. Treatments used in the experiment

	Nomenclature	Description
1	Control	Sterile distilled wáter
2	PEG	Solution of PEG 6000 15 %
3	PEG +1 ESp-1	Solution of PEG 6000 15 % + 1 µL mL^{-1} ESp-1
4	PEG+ 0.5 ESp-1	Solution of PEG 6000 15 % + 0,5 µL mL^{-1} ESp-1
5	PEG+ 0.1 ESp-1	Solution of e PEG 6000 15 % + 0,1 µL mL^{-1} ESp-1
6	PEG +1 ESp-2	Solution of PEG 6000 15 % + 1 µL mL^{-1} ESp-2
7	PEG+ 0,5 ESp-2	Solution of PEG 6000 15 % + 0,5 µL mL^{-1} ESp-2
8	PEG+ 0,1 ESp-2	Solution of PEG 6000 15 % + 0,1 µL mL^{-1} ESp-2

- Final germination percentage (G %)

$$G\,\% = \left(\frac{Gf}{N}\right)100$$

Where:

Gf is the total number of seeds germinated at the end of the test and N is the total number of seeds used in the test.

- Germination speed (VG)

$$VG = \Sigma\left(\frac{Ni}{Ti}\right)$$

Where:

Ni is the number of seeds germinated on day t and Ti is the number of days since the beginning of the germination test.

- Mean germination time (MGT)

$$MGT = \frac{\sum(Ti \times ni)}{\sum ni}$$

Where:

ni is the number of newly germinated seeds at time Ti and T is the number of days from the beginning of the germination test.

- Germination rate (GR)

$$GR = \frac{\Sigma Ni}{\Sigma Ti} Ni$$

Where:

Ni is the number of newly germinated seeds at time Ti.

Statistical processing

The data obtained, in both experiments, were processed by calculating means, standard deviation and confidence intervals at α=0.05.

RESULTS AND DISCUSSION

The results of the final germination percentage of corn seeds cv. P7928 subjected to simulated water deficit with PEG are shown in Figure 1. As can be seen, there were no significant differences in this indicator, neither with PEG, nor with the application of the different concentrations of the extracts evaluated, in either of the two replicates of the experiment.

Previously, it had been found that the final germination percentage of corn seeds was not affected up to -0.9 MPa, and even at -0.3 MPa the highest value of this indicator was obtained (17). The results of the present work confirmed the above, since the osmotic potential used was -0.295 MPa. However, other authors found a decrease in the final percentage of germination in seeds of several maize accessions grown in 10 % PEG solutions, this decrease being more significant at concentrations of 15 and 20 %, although the effect varied with the different cultivars (5).

Table 2 shows the results of the growth indicators of the corn seedlings in both replications, in which it can be observed that the imposed water deficit only significantly reduced the length of the stem. However, in the first replicate there was a significant reduction in root length, an effect that was not observed in the second replicate. Dry mass was not affected in either of the two replicates.

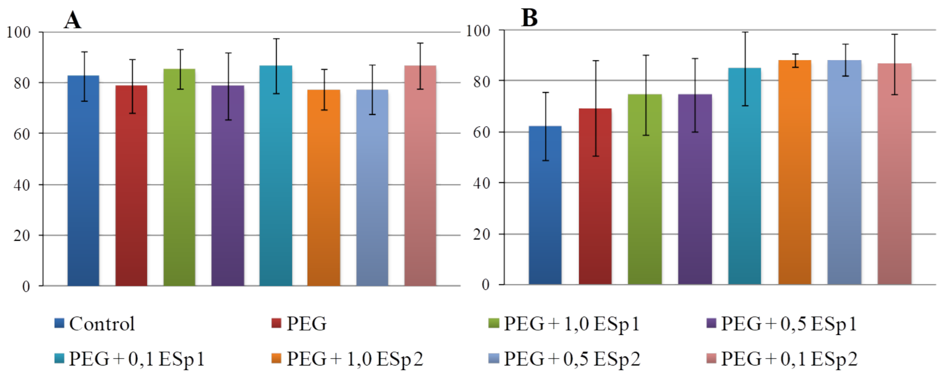
The addition of different concentrations of the two spirulina extracts showed that, in the first replicate, only ESp2 0.1 µL mL^{-1} was able to partially and totally reverse the PEG-induced reductions in stem and root length, respectively. However, it was observed that the concentration 0.5 µL mL^{-1}, of both extracts, was able to partially reverse the PEG-induced reduction in root length; while ESp1 0.1 µL mL^{-1} extract significantly decreased plant length.

In the second replicate, all tested concentrations of both extracts partially reversed the PEG-induced reduction in shoot length and also significantly stimulated root length compared to the control treatment plants, except for ESp2 0.1 µL mL^{-1}.

Dry mass, which was not affected by PEG in either trial, was significantly increased in the first replicate with ESp1 1.0 and 0.1 µL mL^{-1}, however, these treatments significantly decreased shoot length and root length at the lower concentration compared to the control PEG treatment.

The response in the second replicate was different, as all concentrations of both extracts were able to significantly stimulate plant length as well as dry mass, except for the ESp2 0.1 µL mL^{-1} treatment, which only stimulated shoot length.

It is interesting to note that the best response was obtained with the spirulina extract ESp2, in which 70 % ethanol was used as solvent and the maceration lasted 10 days, which would favor its production on a larger scale, since it would be obtained in less time and with less ethanol.



A. First repetition. B. Second repetition (Error bars represent confidence intervals at α=0.05)

Figure 1. Influence of different concentrations of two ethanolic extracts of spirulina on the final germination percentage of corn seeds cv. P-7928 subjected to simulated water deficit with PEG-6000 (15 %)

Table 2. Influence of different concentrations of two alcoholic extracts of spirulina on growth indicators of maize seedlings, cv. P-7928 subjected to simulated water deficit with PEG 6 000 (15 %)

Treatments	Stem length (cm)		Root length (cm)		Dry mass of seedlings (mg)	
	First repetition	Second repetition	First repetition	Second repetition	First repetition	Second repetition
Control	12.0 ± 0.4	9.7 ± 0.4	15.8 ± 0.7	7.8 ± 0.5	257.8 ± 8.6	262.1 ± 2.6
PEG	4.8 ± 0.2	2.0 ± 0.2	12.5 ± 0.5	8.4 ± 0.5	252.8 ± 5.6	256.3 ± 3.1
PEG + 1.0 ESp1	**3.6 ± 0.2***	**4.8 ± 0.3***	12.1 ± 0.4	**10.8 ± 0.7***	**270.0 ± 4.2***	**275.8 ± 3.0***
PEG + 0.5 ESp1	**3.7 ± 0.1***	**4.2 ± 0.2***	**14.0 ± 0.6***	**9.7 ± 0.5***	256.5 ± 6.0	**272.8 ± 5.2***
PEG + 0.1 ESp1	**3.7 ± 0.2***	**4.6 ± 0.2***	**9.9 ± 0.4***	**10.7 ± 0.5***	**301.1 ± 7.8***	**310.3 ± 1.3***
PEG + 1.0 ESp2	4.9 ± 0.1	**4.4 ± 0.1***	12.1 ± 0.6	**13.5 ± 0.6***	252.6 ± 8.9	**278.9 ± 1.2***
PEG + 0.5 ESp2	4.7 ± 0.1	**4.4 ± 3.1***	**13.9 ± 0.6***	**13.5 ± 0.6***	**279.3 ± 6.0***	**284.8 ± 3.1***
PEG + 0.1 ESp2	**5.2 ± 0.1***	**3.1 ± 0.2***	**15.5 ± 0.6***	8.3 ± 0.6	239.0 ± 8.3	240.5 ± 6.2

(Means ± confidence intervals). * Represents treatments that differ significantly from PEG treatment according to confidence interval for α=0.05

The effect observed with ESp1 is probably due to a higher concentration of active compounds extracted, since the longer time in contact with the solvent at a higher concentration (90 %), it could have a higher content of compounds with hormonal action. In this regard, several authors have suggested that seaweed extracts at low concentrations (dilution 1:1000 or more) stimulate positive responses in treated plants, while relatively high concentrations produce inhibitory effects (18, 19). For example, some authors demonstrated that, in wheat plants, mass and length increased with the lowest doses of a *Chorella* extract, while the highest dose had an inhibitory effect. Likewise, the form of application influenced the response, since treatment to *Lepidium sativum* seeds for 24 hours inhibited the length of the aerial part and roots, while addition to the germination medium stimulated these indicators. However, in wheat, seed treatment showed the best results (20).

Recently, the use of *Arthrospira platensis* extracts has been reported to mitigate the adverse effects of water and osmotic stress on plants. Thus, it was shown that treatment of wheat seeds of a tolerant and a susceptible cultivar with an aqueous extract of spirulina (10 %) was able to significantly improve plant vigor, photosynthetic rate, carbohydrate content, and yield components of both cultivars under drought conditions (21).

Previously, in cotton cultivation, it was reported that the application of a spirulina extract stimulated plant tolerance to water stress in salt-affected soils through increased accumulation of nonstructural carbohydrates that improved energy storage and leaf water retention. This is due to the high K^+ content present in the extract, which is necessary to maintain photosynthesis balance under salt and water stress (22).

The effectiveness of the use of spirulina extracts to improve the performance of *Vicia faba* plants subjected to NaCl 135 mmol L^{-1} has also been demonstrated (23). Similarly, the application of aqueous extracts of *Arthrospira platensis* to wheat plants irrigated with seawater increased their antioxidant capacity by increasing antioxidant metabolites and enzymes (16). On the other hand, the application of biochar to the soil and foliar spraying with an aqueous extract of spirulina stimulated the growth of basil plants grown under saline conditions (24).

The results obtained in the first experiment led to the choice of the ESp 2 extract to study the germination dynamics of maize seeds in the presence of water deficit simulated by PEG.

Figure 2 shows the germination dynamics of maize seeds cv. P-7928 subjected to simulated water deficit with PEG-6000. As can be seen, water stress caused a delay in seed germination, since the PEG treatment differed significantly from the other treatments at 24 and 36 h.

After 48 h, no significant differences were observed in any of the treatments evaluated. All three ESp2 concentrations tested were able to reverse the impact of water deficit on germination delay.

Similar results were reported previously, where the osmotic pressure of -0.3 MPa delayed maize seed germination up to 48 h. However, from -0.6 MPa onward, germination percentage was irreversibly affected (25).

Figure 3 shows the results of several germination-related indices. PEG-induced water stress significantly decreased germination velocity (VG) and germination rate (TG), with an increase in mean germination time (MGT). Similar results were found in this same crop with -0.3MPa osmotic pressure induced with PEG (25). Similarly, in maize hybrid lines, increasing the osmotic pressure of the medium with PEG decreased the final percentage and speed of germination (26).

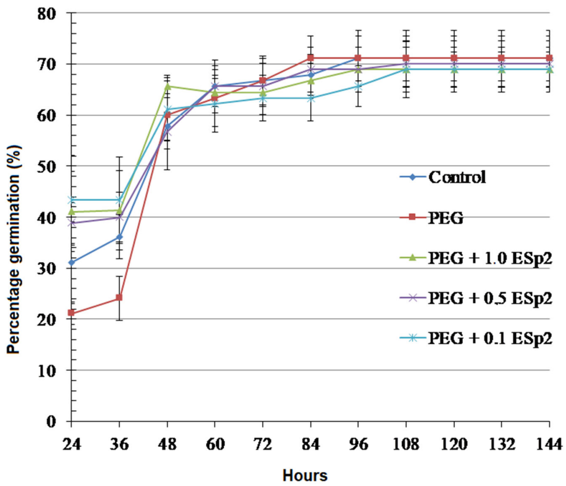
The negative effects are related to the osmotic stress induced by polyethylene glycol that creates a reduction in the solute potential of the medium and, therefore, in the water potential. The decrease in water potential affects the imbibition process and the availability of water to the seeds, which is essential for the hydration of enzymes and substrates involved in the various biochemical reactions that initiate the germination process (27).

Regarding the effect of Spirulina extract on germination rates, concentrations of 1 and 0.1 µL mL^{-1} increased the speed and rate of germination, as well as decreased the MGT.

In this same culture, under normal conditions, the application of *Chorella* sp. increased the germination percentage and decreased the MGT, both in the seeds embedded in the extract and with the application of the extract to the substrate (28).

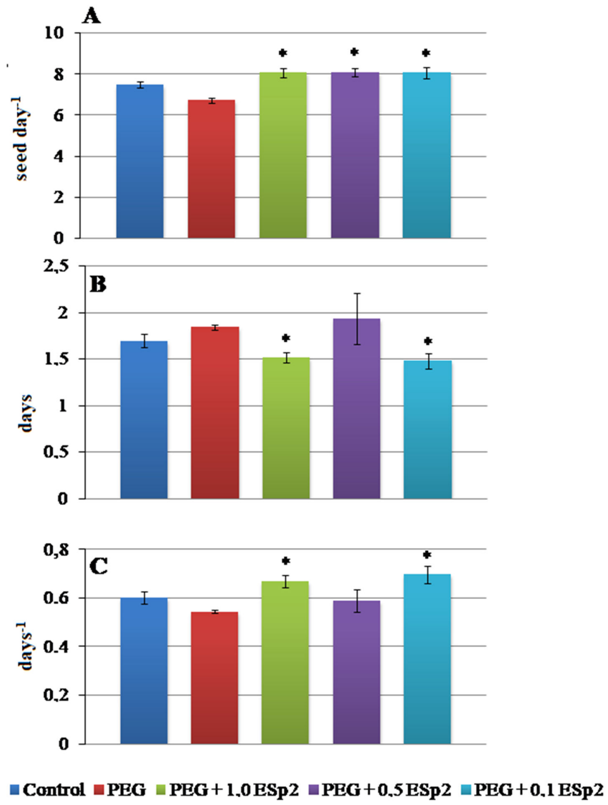
Microalgae and their extracts are natural stimulants that accelerate seed germination and increase seedling vigor when relatively low doses are used (13). Several studies describe their beneficial effects on germination percentage, rate and mean germination time, as well as on plumule and radicle length. These results are attributed to the activation of key enzymatic pathways for germination physiology. For example, α-amylase is an enzyme synthesized in the aleurone layer and its gene expression is regulated by gibberellins. This enzyme is responsible for the mobilization of reserve substances, such as starch, from the endosperm to support embryo growth and differentiation (29). In support of this hypothesis, recently, it was shown that treatment of mung bean seeds for three hours with a Spirulina extract increased gibberellic acid and α-amylase enzyme activity (30). Similarly, several authors have reported decreased protein and carbohydrate contents in peanut, bean, and corn seeds, which correlated with increases in protease and α- and β-amylase enzyme activities (13, 31, 32).

This positive effect of Spirulina extract on the germination of corn seeds subjected to PEG-6000 could contribute to increase their productivity under water deficit conditions. This hypothesis will be investigated and tested in future work.



Error bars represent confidence intervals at α=0.05 and asterisks represent treatments that differ significantly from their respective control

Figure 2. Influence of different concentrations of an alcoholic extract of spirulina on the germination dynamics of maize seeds cv. P-7928 subjected to simulated water deficit with PEG-6000 (15 %)



A. Germination velocity (GV). B. Mean germination time (MGT). C. Germination rate (GR). Error bars represent confidence intervals at α=0.05 and asterisks represent treatments that differ significantly from the PEG treatment

Figure 3. Influence of an alcoholic extract of Spirulina on germination indicators of corn seeds cv. P-7928, subjected to simulated water deficit with PEG-6000 (15 %)

CONCLUSIONS

The simulated water deficit with PEG 6 000 (15 %) delayed the germination of maize seeds cv. P-7928 the first 36 h and reduced the stem length of seedlings, although it did not affect the final germination percentage. The addition of ethanolic extracts of spirulina, in general, partially reversed the decrease in stem length and stimulated root length and seedling dry mass; the extract obtained by maceration with EtOH 70 % for 10 days (ESp 2), at a concentration of 1 µL mL^{-1}, which also completely reversed the delay in germination imposed by PEG, stood out.

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