HORTICULTURA

Seed priming to enhance spinach (*Spinacia oleracea* L.) germination at supraoptimal temperature

Priming de semillas para mejorar la germinación de espinaca (*Spinacia oleracea* L.) a temperatura supraóptima

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ABSTRACT

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Spinach germination is affected by soaking temperature above 20 °C. An experiment was conducted to study the effect of different priming treatments on germination of spinach at 15 and 20 °C. Two osmotic agents for priming were studied: $CaCl_2$ and polyethylene glycol 6000 (PEG). Biopriming with *Azospirillum brasliense* bacteria (3×10^8 UFC mL^{-1}) was performed on half of seeds previously primed with osmotic agents. Three spinach cultivars were used in the experiments: Amadeo, Super Dorrego and Ibis. The experimental layout was completely randomized with five replicates per treatment. At 15 °C germination percentage (GP) of primed seeds increased by approximately 30 %. At 20 °C primed seeds increased GP more than 50 %. At both germination temperatures, biopriming did not allow a greater GP increase compared to seeds only primed with osmotic agents.

Keywords: *Azospirillum brasilense*; biopriming; halopriming; heat stress; osmopriming.

RESUMEN

Breit, A.; Larraburu, E. E. & Castañares, J. L. (2025). Priming de semillas para mejorar la germinación de espinaca (*Spinacia oleracea* L.) a temperatura supraóptima. Horticultura Argentina 44 (113): 24-31. https://id.caicyt.gov.ar/ark:/s18519342/z3ebj91lk

La germinación de la espinaca es inhibida cuando la temperatura es superior a 20 °C. Se realizó un experimento para estudiar el efecto de diferentes tratamientos de priming sobre la germinación de espinaca a 15 y 20 °C. Se estudiaron dos agentes osmóticos para el priming: $CaCl_2$ y polietilenglicol 6000 (PEG). Se realizó un biopriming con bacterias *Azospirillum brasliense* (3×10^8 UFC mL^{-1}) en la mitad de las semillas previamente tratadas con agentes osmóticos (priming). En los experimentos se utilizaron tres cultivares de espinaca: Amadeo, Super Dorrego e Ibis. El diseño experimental fue completamente aleatorizado, con cinco repeticiones por tratamiento. A 15 °C, el porcentaje de germinación (PG) de las semillas tratadas con los agentes osmóticos aumentó aproximadamente un 30 %. A 20 °C, las semillas sometidas a priming con agentes osmóticos aumentaron el PG en más del 50 %. En ambas temperaturas de germinación, el biopriming no condujo a un aumento del PG en comparación con las semillas sometidas a priming.

Palabras claves: *azospirillum brasilense*; biopriming; halopriming; estrés térmico; osmopriming.

1. Introduction

Spinach (*Spinacia oleracea* L.) is an annual crop cultivated during cool season, with optimal temperatures between 15 and 18 °C (Uçgun *et al*., 2020). Germination is affected by soaking temperatures above 20 °C (Chitwood *et al*., 2016; Magnée *et al*., 2020). This can lead to a delay or inhibition of germination or affect uniformity (Imran *et al*., 2021). The main causes of decrease in germination could be associated with the physical restriction of the pericarp and secretion of chemical inhibitors (such as abscisic acid, ABA) promoted at supraoptimal temperatures (Ziaf *et al*., 2017).

Techniques studied to improve germination at supraoptimal temperatures include pericarp removal (Leskovar *et al*., 1999), acid scarification (Masuda *et al*., 2005), seed soaking with $NaOCl/H_2O_2$ (Katzman *et al*., 2001) and seed priming (Anjos Neto *et al*., 2020; Chen *et al*., 2010).

Seed priming is a pre-germinative treatment discovered by Heydecker *et al*. (1975) and consists of a controlled rehydration of seeds that allows the occurrence of metabolic processes typical of the first phases of germination, without reaching this stage, and results in a better response of seeds and plants to adverse conditions (Paul *et al*., 2022; Paparella *et al*., 2015). In these phases, several metabolic and repairing events at the cellular level occur (Devika *et al*., 2021) which will have a longer time to occur. Primed seeds stay in a ´germinating state´ with improved germination rate and uniformity under optimal and adverse conditions (Zulfiqar, 2021). It has been widely studied as a low-cost technique to increase crops tolerance against different types of stress (Liu *et al*., 2022; Singh *et al*., 2020).

There are several types of seed priming according to the way in which seeds hydration is controlled. The most common technique consists in the use of agents that reduce the osmotic potential of the medium in which seeds are hydrated, limiting water absorption. These osmotic agents can be inorganic salts (´halopriming´) or sugars or polyethylene glycol (´osmopriming´) (Sher *et al*., 2019). Additionally, some priming techniques combine controlled imbibition with the addition of agents such as mineral nutrients (´nutrient priming´), growth regulators (´hormonal priming´) or beneficial microorganisms (´biopriming´) (Jisha *et al*., 2013).

The objective of this study was to evaluate the effect of seed priming with two osmotic agents, $CaCl_2$ and polyethylene glycol (halopriming and osmopriming, respectively), and *Azospirillum brasilense* bacteria (biopriming) on spinach germination at optimal and supraoptimal temperatures.

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2. Materials and methods

The experiment was conducted between february and august 2024 in the Plant Physiology Laboratory of the National University of Lujan. Three spinach cultivars were used: Amadeo (INTA), Super Dorrego (Advan Seed) and Ibis (Rijk Zwaan).
Two osmotic agents were evaluated for halopriming and osmopriming treatments: $CaCl_2$ and polyethylene glycol 6000 (PEG), respectively. The concentration of these agents was adjusted to reach an osmotic potential (Ψo) of -1.3 MPa, according to van't Hoff equation (Shabala & Munns, 2017) for $CaCl_2$ and Michel & Kaufmann (1973) equation for PEG.
Priming was performed in a culture chamber (Semedic I-500 PF) at 13 °C (± 0.5°C), 45% RH and darkness. Seeds were placed between two filter paper (Whatman No 1), in 8.5 cm diameter Petri dishes, and soaked with the priming solution in a ratio of 1:5 (weight:volume).
After eight days, seeds were removed and rinsed in distilled water. Half of the group of primed seeds were immersed in a suspension of *Azospirillum brasilense* AZ 39 during 30 min (biopriming*)*. In addition, a group of seeds was treated only with the bacteria. The Az 39 titer was adjusted to 3×10^8 UFC mL^{-1}, obtained at exponential growth phase according to (Rodríquez Cáceres, 1982).
Then, seeds were put to germinate between two filter paper, soaked with distilled water in a ratio of 1:5 (weight:volume).
Seeds were separated into two groups in germination chambers (Semedic I-500 PF) with different temperatures: 15 and 20 °C (± 0.5°C), 45% RH and dark. Non-primed seeds (control) were put to germinate in both temperatures. Germination was recorded daily for 10 days. Seeds were considered germinated when the radicle was visible. Germination percentage (GP) was calculated according to the following formula: GP (%) = (n/N) × 100, where n is the number of seeds germinated during the experiment and N is the total number of seeds (ISTA, 2013).
The experimental layout was completely randomized. All values reported were the means of five replicates and the data were analyzed using Tukey's test at 0.05 confidence level with Infostat statistical software package (Di Rienzo *et al*., 2011).

3. Results

At 15 °C, optimal spinach germination temperature, an increase in GP of 15 to 20 % was measured in all priming treatments, compared to untreated seeds (Fig. 1). There was no increase in germination in seeds inoculated with *Azospirillum brasilense* after priming. Seeds only treated with *Azospirillum brasilense* did not improve germination compared to those without treatment.
The negative effect of higher temperatures (20 °C) was evident due to the low germination of control treatments (Fig. 2), in which GP was between 15 and 40 %. Seed priming increased GP to values between 75 and 90 %. As in the experiment at 15 °C, treatment with *Azospirillum brasilense* did not increase germination in any case.

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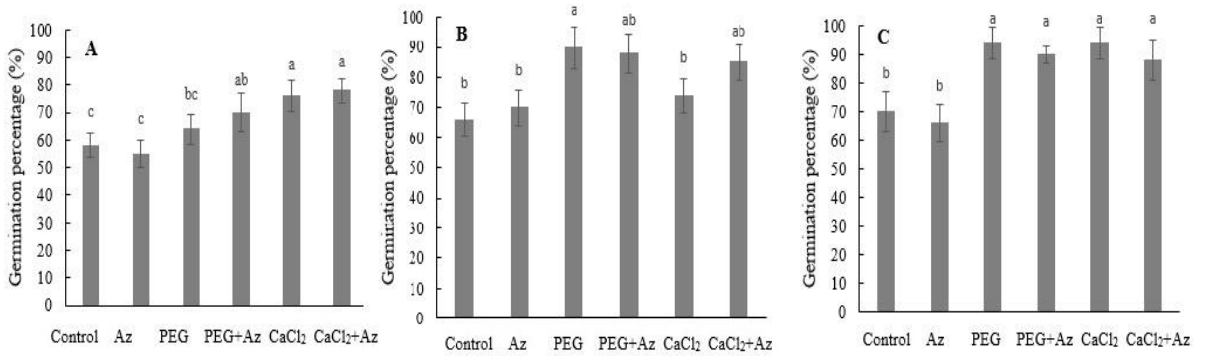

Figure 1: Effect of different priming treatments on germination percentage at 15 °C of spinach seeds Amadeo (A), Super Dorrego (B) and Ibis (C) cultivar. [Control: non-primed seeds; Az: seeds only treated with *Azospirillum brasilense*; PEG: seeds primed with PEG; PEG+Az: seeds treated with *Azospirillum brasilense* after priming with PEG; $CaCl_2$: seeds primed with $CaCl_2$; $CaCl_2$+Az: seeds treated with *Azospirillum brasilense* after priming with $CaCl_2$.] Vertical bars represent ± S.E. Different letters indicate a significant difference ($p \leq 0.05$) according to Tukey. Luján, Buenos Aires, Argentina, 2024.

Figura 1: Efecto de diferentes tratamientos de priming en el porcentaje de germinación a 15 °C de semillas de espinaca, en los cultivares Amadeo (A), Super Dorrego (B) y Ibis (C). [Control: semillas no sometidas a priming; Az: semillas tratadas solamente con *Azospirillum brasilense*; PEG: semillas sometidas a priming con PEG; PEG+Az: semillas tratadas con *Azospirillum brasilense* luego del priming con PEG; $CaCl_2$: semillas sometidas a priming con $CaCl_2$; $CaCl_2$+Az: semillas tratadas con *Azospirillum brasilense* luego del priming con $CaCl_2$.] Las barras verticales representan ± D.E. Letras diferentes indican una diferencia significativa ($p \leq 0,05$) de acuerdo a Tukey. Luján, Buenos Aires, Argentina, 2024.

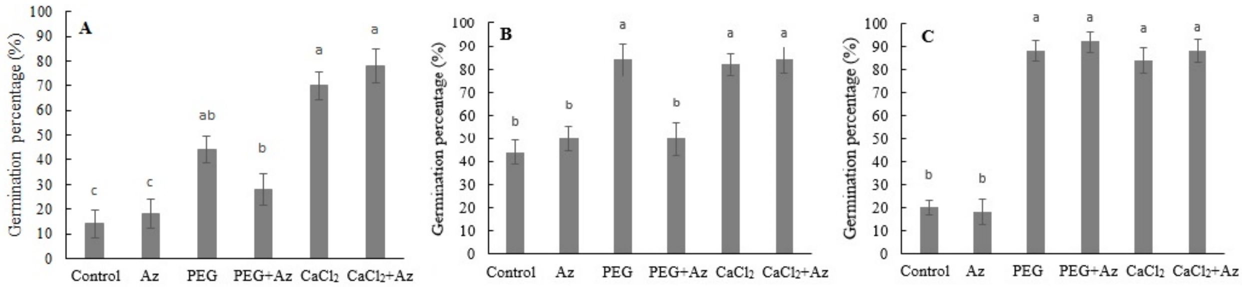

Figure 2: Effect of different priming treatments on germination percentage at 20 °C of spinach seeds Amadeo (A), Super Dorrego (B) and Ibis (C) cultivar. [Control: non-primed seeds; Az: seeds only treated with *Azospirillum brasilense*; PEG: seeds primed with PEG; PEG+Az: seeds treated with *Azospirillum brasilense* after priming with PEG; $CaCl_2$: seeds primed with $CaCl_2$; $CaCl_2$+Az: seeds treated with *Azospirillum brasilense* after priming with $CaCl_2$] Vertical bars represent ± S.E. Different letters indicate a significant difference ($p \leq 0.05$) according to Tukey. Luján, Buenos Aires, Argentina, 2024.

Figura 2: Efecto de diferentes tratamientos de priming en el porcentaje de germinación a 20 °C de semillas de espinaca cultivares Amadeo (A), Super Dorrego (B) y Ibis (C). [Control: semillas no sometidas a priming; Az: semillas tratadas solamente con *Azospirillum brasilense*; PEG: semillas sometidas a priming con PEG; PEG+Az: semillas tratadas con *Azospirillum brasilense* luego del priming con PEG; $CaCl_2$: semillas sometidas a priming con $CaCl_2$;

$CaCl_2$+Az: semillas tratadas con *Azospirillum brasilense* luego del priming con $CaCl_2$.] Las barras verticales representan ± D.E. Letras diferentes indican una diferencia significativa ($p \leq 0,05$) de acuerdo a Tukey. Luján, Buenos Aires, Argentina, 2024.

4. Discussion

Priming conditions were chosen based on Chen *et al*. (2010), who evaluated different priming osmotic potential (using PEG 8000), temperature and duration for spinach cv. Bloomsdale, and concluded that the best germination at high temperature was achieved after priming at -6.0 MPa, 13 °C and eight days duration. However, in our experiments we worked with -1.3 MPa osmotic potential since it has been proposed as the ideal for spinach seed priming by other researchers (Atherton & Farooque, 1983; Masuda *et al*., 2005).
Respect to the osmotic agents selected, PEG is one of the most common because is chemically inert, non-damaging to proteins and its large molecular size prevents diffusion into seeds, avoiding cytotoxic effects (Lei *et al*., 2021). Nevertheless, PEG solutions are highly viscous, so it can affect oxygen diffusion and sometimes are difficult to completely remove from seeds after treatment (Srivastava *et al*., 2022). Inorganic salts, like $CaCl_2$ used in our experiments, do not have these limitations and their cost is usually lower. In our experiments, although a positive priming response was observed at supraoptimal temperatures, no differences were found between PEG and $CaCl_2$ (Fig. 2).
Our results are consistent with those reported by (Chen *et al*., 2010), who increased spinach germination at 20 °C in primed seeds with PEG. The beneficial effects of seed priming on germination at high temperatures was also measured in carrot (*Daucus carota* L.) (Pereira *et al*., 2009), maize (*Zea mays* L.) (Mahboob *et al*., 2015) and rice (*Oryza sativa* L.) (Kata *et al*., 2014).
When comparing osmotical agents, PEG and $CaCl_2$, in general, no differences were observed at either temperature. This contrast with what was reported by Castañares (2019), who evaluated the effect of priming lettuce (*Lactuca sativa* L.) seeds with PEG, KNO_3 and $CaCl_2$ on germination at supraoptimal temperatures, and the best response was observed with this last salt. Similar results were obtained in pansy (*Viola x wittrockiana*) seeds primed with PEG and several salts and put to germinate at high temperatures (Yoon *et al*., 1997).
Seed biopriming is a technique which consists in seed inoculation with beneficial microorganisms along with seed-controlled hydration (Mahmood *et al*., 2016). This treatment allows beneficial microorganisms to establish on the seed surface providing protection against different adverse conditions (Singh *et al*., 2023). *Azospirillum* is an important genus of beneficial microorganisms whose advantages as a biopriming agent has been reported (Chakraborti *et al*., 2022; Fiodor *et al*., 2023), improving response against biotic and abiotic stress. In rice (*Oryza sativa* L.), Raja *et al.* (2017) compared the effect of different bacteria for biopriming treatment and the best response in germination percentage and speed of germination was measured with *Azospirillum*.Liu *et al.* (2018), increased germination of tall fescue (*Festuca arundinacea* Schreb.), orchardgrass (*Dactylis glometata* L.) and russian wildrye (*Psathyrostachys juncea* (Fisch.) Nevski) aged seeds primed with H_2O_2 and *Azospirillum brasilense.* In our experiments, although a positive response was observed in bioprimed seeds, these treatments did not differ significantly from those primed only with osmotic agents (Fig. 2). On the other hand, seeds treated only with bacteria did not differ from control treatment. Therefore, in our experimental conditions, it is not possible to deduce a beneficial effect of biopriming.

5. Conclusions

Spinach germination is highly affected by temperatures above 20 °C. Therefore, any treatment for reaching adequate germination in such conditions will be of great interest. Under our experimental conditions, the beneficial effect of priming with osmotic agents in improving germination at supraoptimal temperatures was evident. On the other hand, a greater increase in germination was not possible when combining osmo and halopriming with biopriming with *Azospirillum brasilense*. It would be interesting to carry out other investigations evaluating different promoting bacteria, as well as concentrations, times and durations of treatment.

6. Conflict of interests

The authors declare that this work does not present conflict of interests.

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