BIO Web of Conferences **205**, 01010 (2025) https://doi.org/10.1051/bioconf/202520501010
EE&AE 2025

Seed priming with boron-based nanoparticles mitigates tomato losses in hyperthermia stress

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Abstract. Global climate change can increase the frequency and severity of hyperthermia stress, which critically affects seed germination, a process essential to crop production. In this study, we investigated the potential of bare boron nanoparticles (B NPs) and B NPs coated with polyvinylpyrrolidone (B-PVP NPs) at 0.1, 1, and 10 mg/L as seed priming agents for tomato (Solanum lycopersicum) to mitigate losses caused by hyperthermia-induced stress (+45° C). Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR) confirmed that B-based NPs were in B2O3 chemical composition with an irregular morphology, as well as the presence of PVP. Seed priming with B NPs increased germination viability through rate, potential, and index, while B-PVP NPs performed more efficiently at lower concentrations, although mean germination time remained unchanged in all treatments. Principal Component Analysis revealed that over 76% of the variance in germination is explained by rate, potential, and index. These results can be explained by boron being an essential nutrient crucial for wall integrity and cell membrane stability. This suggests that B-based nanopriming of tomato seeds can mitigate losses caused by hyperthermia, serving as a sustainable strategy to enhance tomato resilience under climate-induced stress conditions.

1 Introduction

Global climate change is one of the biggest threats to agriculture, jeopardizing food security. Extreme temperature events can cause hyperthermia stress due to elevated temperatures during early developmental stages, significantly impairing seed germination and seedling establishment [1].

Tomato (*Solanum lycopersicum*), a globally cultivated horticultural crop, is especially vulnerable to hyperthermia stress, which can reduce germination viability and emergence rates [2]. To counteract the detrimental effects of abiotic stress, nanotechnology can be applied in agriculture by using nanomaterials as nanofertilizers to enhance seed germination and metabolic activities. These nanofertilizers can be delivered through different methods, such as root-soil, foliar applications, and seed priming [3]. Amongst the delivery systems, seed priming can be more efficient as nanofertilizers than other methods in tomato, as observed in a previous study [4].

Seed priming is the process of delivering the nanomaterials to the seed prior to germination. In tomato, seed priming has been used with different nanofertilizers such as gibberellic acid, Selenium, and Zinc [5]. Boron is an essential micronutrient for higher plants,

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related to cell wall integrity, membrane function, and reproductive development [6]. The application of Boron-based nanoparticles (B NPs) can allow controlled delivery and enhance their bioavailability. Furthermore, surface modification with polyvinylpyrrolidone (B-PVP NPs) can improve nanoparticle dispersion, enhance colloidal stability, and modify their growth [7].

In this context, the present study evaluates the effects of seed priming with bare B NPs and PVP-coated B NPs (B-PVP NPs) for tomato under hyperthermia stress conditions (+45°C). Understanding the potential of B-based NPs to mitigate losses of tomato under high-temperature-induced damage could offer a sustainable approach to climate-resilient crop establishment.

2 Materials and methods

2.1 Characterization of B-based nanoparticles

PVP-coated and uncoated Boron-based nanopowders (99.9% purity, 100 nm; US Nanomaterials, Inc.) were dispersed in Milli-Q water at concentrations of 0.1, 1, and 10 mg/L. B-based NPs were analyzed via Transmission Electron Microscopy (TEM) following standard procedures, and Fourier Transform Infrared Spectroscopy (FTIR) [8].

2.2 Seed priming and hyperthermia exposure

Commercial tomato seeds (Var. Floradade, U.S.A.) were surface sterilized with NaClO (10 min, 8%) and exposed for 8 hours to seed priming treatments. After nanopriming, seeds were sown in germination micro-greenhouses with 24 tomato seeds in quadruplicates. Seeds were kept in darkness for 5 days; on the 5th day, the micro-greenhouses were closed and exposed to the sun, allowing for a heat-stress event to be replicated. The temperature reached +45°C and was maintained for 4 hours. Then, the micro-greenhouses were opened and kept away from the sun, allowing the temperature to normalize and the seeds and seedlings to continue development for 2 more days.

2.3 Determination of germination viability

The viability of germination was determined according to standard methods [9].

On the 5th day, the germination potential was determined (germinated seeds at 5th day/total seeds in the experiment) * 100%.

On the 7th day, the germination rate was evaluated (germinated seeds/total seeds in the experiment) * 100%. The germination index was calculated (Σ seeds germinated per day/days of germination). The mean germination time was measured (Σ seeds germinated per day * number of days since the start of the experiment) / total seeds in the experiment.

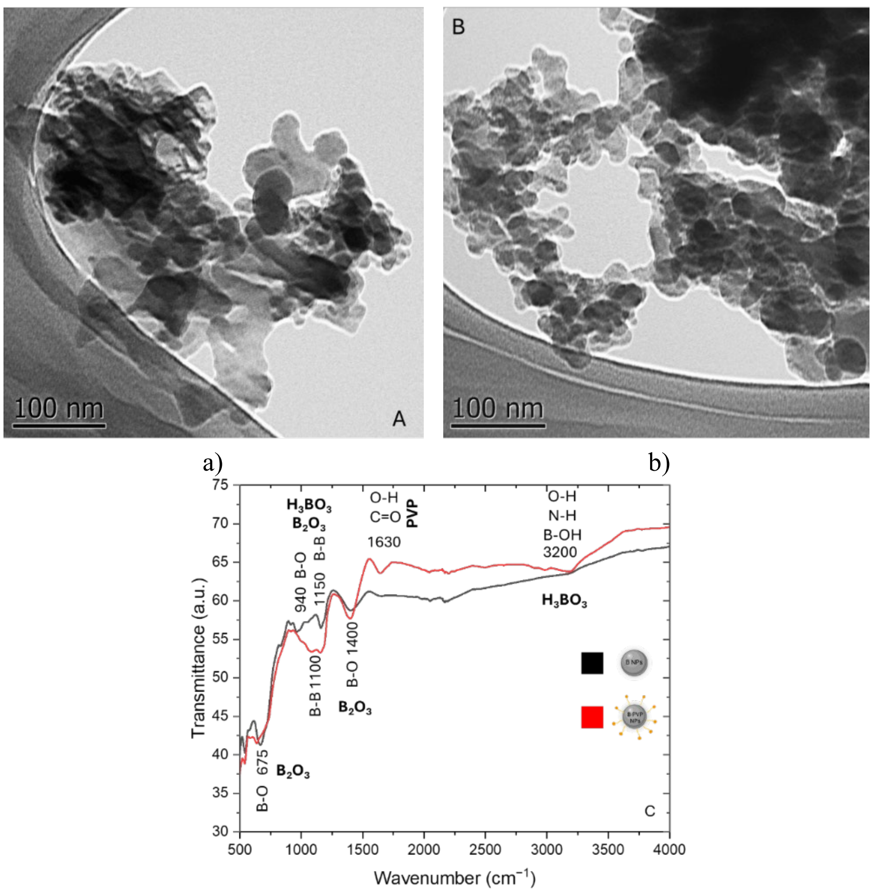
2.4 Statistical analyses

The germination viability parameters, namely germination potential, rate, index, and mean germination time, were analyzed using a two-way ANOVA with Origin Pro 2024b Student Ed. The first factor was the surface modification (PVP coating), and the second factor was the concentration (0.1, 1, and 10 mg/L). A Principal Component Analysis (PCA) was also conducted with the germination viability parameters.

3 Results and Discussion

3.1 Characterization of B-based nanoparticles

We confirmed the presence of electron-dense nanomaterials through TEM micrographs. Both B-based NPs had an irregular morphology. The primary size obtained for B NPs (Fig. 1A) was 81.89 ± 21.13 nm, and 121.62 ± 19.12 nm for B-PVP NPs (Fig. 1B). It appears that PVP presence could promote the agglomeration of B-based NPs, as observed in Fig. 1B. This is possible due to PVP's affinity to bind water molecules; therefore, this process could form a hydration layer in the NPs [7]. Moreover, we confirmed that the nanomaterials were mainly composed of B_2O_3 through FTIR (Fig. 1C). The chemical composition of B_2O_3 in the NPs means the dissolution of B-based NPs will be in the chemical form of H_3BO_3, which is the primary form in which plants take up their boron requirements [10]. Therefore, B-based NPs can supply bioavailable B for tomato. The presence of PVP was also confirmed by FTIR, where the characteristic lactam ring and amide functional group were observed (Fig. 1C).



c)

Fig. 1. Characterization of B-based NPs. A) TEM micrograph of bare B NPs. B) TEM micrograph of PVP-functionalized B NPs. C) FTIR spectra of B NPs (black) and B-PVP NPs (red) solid samples.

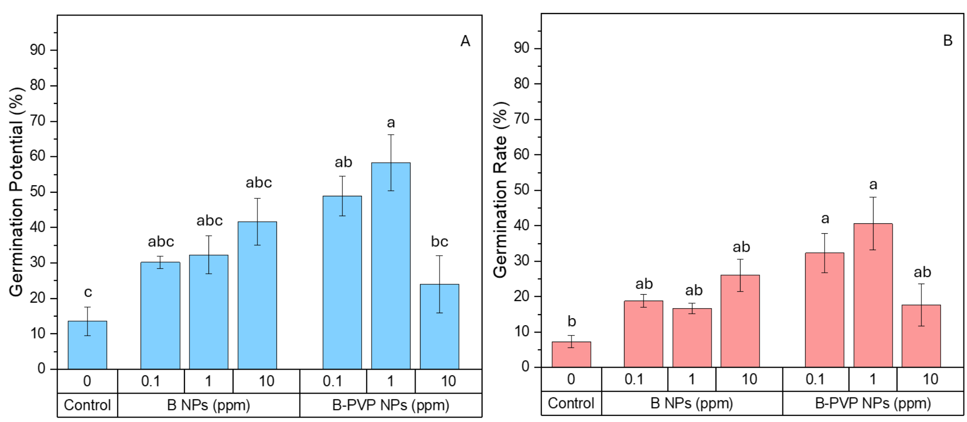
B_2O_3 NPs have also been characterized in other studies. For instance, B_2O_3 NPs were used as antifungal agents and nutrient sources for pea plants, where the NPs, observed by

HR-TEM, had spherical shapes and a size range of 42.10 – 92.89 nm [11]. In a different study, spherical-shaped B_2O_3 NPs were also observed through TEM, where the size ranged from 52 to 89 nm [12].

Moreover, glass NPs containing B_2O_3/ZnO presented absorption bands around 1430 cm^{-1}, where we also observed the characteristic transmittance of B-O asymmetric stretching [13]. The same authors also observed O-H bands in the 1640 cm^{-1} region, attributed to molecular water. We also observed the presence of water in this region, as well as C=O in the lactam ring of PVP. Transmittance bands corresponding to B-O stretch were observed in the 675 and 940 cm^{-1} regions, while B-B stretching was identified around the 1100 – 1150 cm^{-1} region [14].

3.2 Germination viability under hyperthermia exposure

Seed priming with B-based NPs increased the overall germination viability of tomato seeds under hyperthermia stress (Fig. 2). At the 5th day, the germination potential of tomato seeds increased paired with concentration for B NPs (Fig. 2A), where 10 mg/L achieved the highest germination potential, of 207.7% higher than control, whereas 0.1 and 1 mg/L resulted in 123.1 and 138.5%, respectively. This trend was repeated with B-PVP NPs (Fig. 2A), but only for the 0.1 and 1 mg/L treatments, where the germination potential increased by 261.5 and 330.8%, respectively. However, seed priming with B-PVP NPs at 10 mg/L resulted in only a slight increase in germination potential by 76.9% as compared to the control.

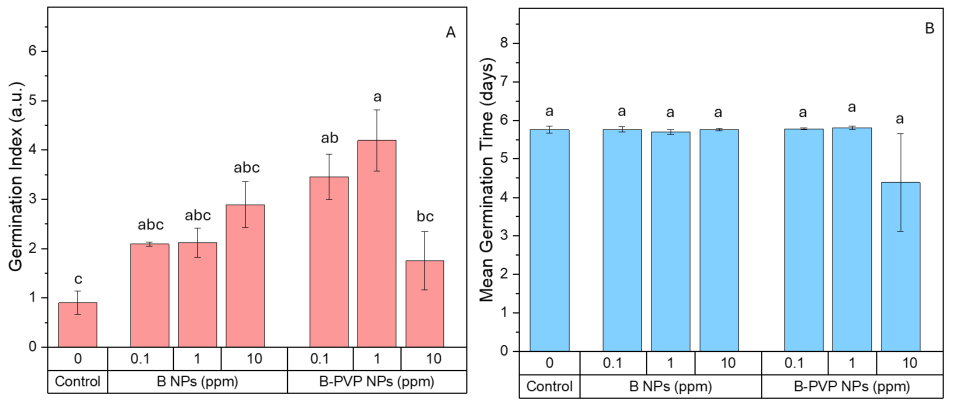


a) b)

Fig. 2. Germination viability of tomato seeds. a) Germination potential in % of tomato seeds exposed to different seed priming treatments of B-based NPs. b) Germination rate in % of tomato seeds exposed to different seed priming treatments of B-based NPs after hyperthermia stress.

After being exposed to hyperthermia, we observed the germination rate of tomato on the 7th day. In all treatments and controls, there is a reduction between 26.1% and 48.4% in the number of seedlings. This reduction is possibly due to physiological, biochemical, and molecular disruptions caused by hyperthermia, which led to the death of seedlings and inhibited the germination of new seedlings. However, the different treatments with both B NPs and B-PVP NPs helped to mitigate these losses (Fig. 2B). For B NPs, the reduction in losses was between 8.22 and 10.9%, although statistical significance was only found with B-PVP NPs, where loss reduction reached up to 12.1% (Fig. 2B).

We also observed similar improvements in the germination index (Fig. 3A) after hyperthermia stress in tomato, where B NPs achieved increases of 132.5 – 221.4%, and B-PVP of 95.2 – 365.9%. The concentration trends in germination potential and germination rate were consistent with the germination index. However, the mean germination time (Fig. 3B) was not changed by any treatment, remaining statistically the same as the control. Therefore, B-based NPs can increase the germination viability of tomato seeds through different parameters, but not the speed of germination.



a) b)

Fig. 3. Germination viability of tomato seeds. a) Germination index in a.u. of tomato seeds exposed to different seed priming treatments of B-based NPs after hyperthermia stress. b) Mean germination time in days of tomato seeds exposed to different seed priming treatments of B-based NPs.

We also conducted a Principal Component Analyses (PCA) of the different germination parameters to confirm the trends observed in germination potential (GP), germination rate (GR), and germination index (GI) (Fig. 4). In this PCA we identified that these parameters correspond to the first principal component (PC 1), which can explain 76.65% of the variance of the results. Furthermore, we also confirmed that mean germination time (MGT) had a different trend, as previously observed in Fig. 3B, explaining a further 21.69% of the variance in the results.

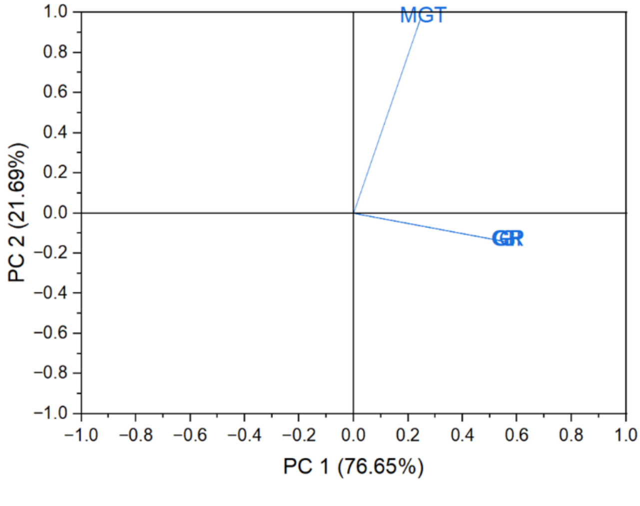


Fig. 4. Principal Component Analysis of germination viability in B-nanoprimed tomato seeds exposed to hyperthermia stress.

The impact of nanopriming tomato seeds with B-based NPs is positive and mitigates the losses under hyperthermia stress. However, the mechanisms by which B-based NPs increase the germination viability are poorly understood.

These results could be explained by the nature of B itself, as an essential micronutrient for tomato crops [6]. B is involved in several physiological and structural processes, such as cell wall cross-linking via borate diester formation with rhamnogalacturonan-II, and plasma membrane integrity [10]. Therefore, during the early stages of germination, adequate B availability could support radicle emergence and cellular division/expansion, potentially increasing the germination viability.

Moreover, delivering B needs by nanopriming tomato seeds might allow for a gradual delivery of B in its bioavailable form as boric acid, which could be utilized by germinated seeds. The enhanced performance of B-PVP NPs at lower concentrations might be attributed to improved dispersion and reduced agglomeration, which could promote the interactions between seed-NP and uptake of B. However, at 10 mg/L, the steric repulsion forces might not be sufficient to prevent the agglomeration of B-PVP NPs, as shown in Fig. 1B, which could reduce the bioavailability of B or induce phytotoxicity due to overaccumulation.

Together, these findings indicate that seed priming with B-based NPs, especially B-PVP NPs at lower concentrations, can serve as an effective strategy to enhance germination of tomato seeds and mitigate losses due to hyperthermia stress. Further physiological, biochemical, and molecular analyses will be needed to unravel the underlying mechanisms involved in interactions between B-based NPs and tomato seeds.

4 Conclusions

This study revealed that seed priming with B-based NPs, such as bare B NPs and B-PVP NPs, can increase the germination viability of tomato seeds under hyperthermic conditions. The mitigation of losses caused by hyperthermia can be attributed to B-based NPs, which increase germination before the heat event, resulting in a higher number of tomato seedlings surviving afterward. However, it appears that both factors analyzed in this study, concentration and surface-functionalization with PVP, can influence the efficiency of B NPs to improve germination viability. It is unclear what mechanisms B NPs use to increase the germination of tomatoes. However, since B is an essential micronutrient, further research is encouraged to elucidate these mechanisms and enable the implementation of sustainable agricultural practices for climate-resilient crop establishment. Future research should include physiological and biochemical analyses to clarify how B-based NPs enhance germination under heat stress. Assessing antioxidant activity, B uptake, and gene expression of stress-related pathways could reveal the mechanisms involved.

EGL thanks SECIHTI and Tecnologico de Monterrey for the scholarship granted (1016970). PCA thanks to SECIHTI (330129). The authors thank Lourdes Palma for technical support in the Microscopy Unit in the Neurobiology Institute at the National Autonomous University of Mexico (UNAM).

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