



# Chemical Seed Priming with Zinc Sulfate Improves Quinoa Tolerance to Salinity at Germination Stage <sup>†</sup>

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<sup>†</sup> Presented at the 2nd International Laayoune Forum on Biosaline Agriculture, 14–16 June 2022; Available online: <https://lafoba2.sciforum.net/>.

**Abstract:** This study was conducted to assess the effect of seed pre-treatment “priming” with zinc sulfate (ZnSO<sub>4</sub>) on the improvement in germination in three quinoa genotypes, “ICBA-Q5”, “Puno” and “Titicaca”, under different salinity levels and to characterize some physiological traits of seed tolerance to salinity. The germination tests were conducted to assess the priming effect on germination. Samples of 50 quinoa seeds of the 3 genotypes were soaked in 1 g/L of ZnSO<sub>4</sub> solution for 8 h and then were dried under ambient temperature. Then, each seed sample was placed in a Petri dish containing filter paper imbibed with a salt solution of 300, 400 and 500 mM NaCl. The numbers of germinated seeds were noted every 24 h and seed samples were collected for reserve mobilization analysis. The results showed that, under control conditions, ICBA-Q5 showed the highest germination percentage, followed by Puno and then Titicaca. The salinity level of 300 and 400 mM NaCl severely inhibited the seed germination in all of the tested genotypes and the concentration of 400 mM NaCl was considered the highest threshold for germination in the quinoa genotypes tested. The priming treatment improved the germination parameters and the improvement was more evident for germination speed and the final germination percentages that were generally increased by ZnSO<sub>4</sub> priming by more than 100% for all of the genotypes.

**Keywords:** priming; quinoa; salinity; germination; zinc sulfate



**Citation:** Bourhim, M.R.; Cheto, S.; Qaddoury, A.; Hirich, A.; Ghoulam, C. Chemical Seed Priming with Zinc Sulfate Improves Quinoa Tolerance to Salinity at Germination Stage. *Environ. Sci. Proc.* **2022**, *16*, 23. <https://doi.org/10.3390/environsciproc2022016023>

Academic Editor: Redouane Choukr-Allah

Published: 16 June 2022

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## 1. Introduction

Salinity is among the most harmful abiotic stresses that causes many disruptions to crop productivity [1–3] consequently affecting plant growth and water accessibility, due to the osmotic stress, the ion toxicity, the oxidative stress and a critical K<sup>+</sup> deficiency [2].

Generally, plant tolerance to such abiotic stresses involves responses at the cellular level until the entire plant levels throughout at the phenological stage, the physiological interactions that occur between plant processes, plant organs and plants and their environment, resulting in the alleviation of the adverse stress and then improvement of crop production [4]. Germination is a crucial stage for crop establishment, and it is very sensitive to any variation in the necessary environmental conditions allowing embryo-development and seedling emergence. For many crops, ranked as tolerant to salt salinity, their seeds were revealed to be sensitive to this constraint at germination. For quinoa, in spite of its tolerance to salinity, its seed germination was severely inhibited particularly with high salinity levels [3]. To ensure high crop establishment under salinity, seed germination needs to be enhanced by some efficient and easily applied pretreatments [5].

Seed priming is a physiological technology that provides an advantage by improving the speed of seed germination, even under abiotic stresses, such as salinity, drought, very

high and very low temperatures, etc. [6]. Priming is also considered as a pre-germinating stress exposure that will create a stress memory as information that improves the plant's physiological processes [2]. Priming is also known as seed preconditioning that has the capacity to modulate the effects of abiotic stresses on crop plants [4].

Our present work aims for the improvement of seeds germination under high salinity levels by some pre-germinative treatments in three genotypes of quinoa (*Chenopodium quinoa* Willd).

## 2. Materials and Methods

### 2.1. Plant Material

In this present experiment, the seeds of three quinoa genotypes "Puno", "Titicaca", and "Q5" were used in this study to test the priming effect on the improvement of seed germination using filter paper in Petri dishes.

### 2.2. Priming Tests and the Germination under Salinity Stress

#### 2.2.1. Determination of Accurate Priming Duration and Zinc Sulfate Concentration

This test was carried out on the genotype with the highest germination percentage in distilled water, in order to determine the efficient concentration of the priming chemical "zinc sulfate" and the optimal time of treatment. Thus, the seeds were soaked in 3 different concentrations (0.1, 0.5 and 1 g/L of zinc sulfate) at 4 durations (4, 6, 8 and 12 h). The germination test was carried out in 9 cm Petri dishes containing filter paper at a density of 50 seeds per dish. The seeds and filter paper were imbibed with 1 mL of 200 mM NaCl solution to ensure the differences between ZnSO<sub>4</sub> concentrations can be easily observed. Additionally, a negative control with no priming treatment and a positive control consisting of germination in distilled water were included.

#### 2.2.2. Effect of Priming with Zinc Sulfate on the Improvement of Germination under Salinity

The experiments were conducted in the laboratory and carried out on three genotypes with the selected concentration of ZnSO<sub>4</sub> (1 g/L) and the treatment time retained from the first test (8 h). The germination process was carried out in 9 cm Petri dishes, as described above. Samples of 50 seeds were placed on each dish, and the filter papers were imbibed with 1 mL of saline solutions consisting of 300, 400 and 500 mM of NaCl. Simultaneously, the negative control (without priming) was prepared for each genotype and saline concentration. Each treatment was repeated three times and the number of germinated seeds was noted each day (24 h).

Some germination parameters as the mean germination time (MGT) and final germination percentage (FGP) were calculated, as described by [7].

$$MGT = \sum(NiTi) / \sum Ni$$

where  $N$  is the number of seeds germinated at time  $i$ , and  $Ti$  is the time (day) from sowing. The value of MGT is inversely proportional to the germination speed.

$$FGP = (\text{Final number of germinated seeds} / \text{Total number of seeds sown}) \times 100$$

### 2.3. Statistical Analysis

The statistical analysis was performed using "SPSS" program version 25. All means values and standard deviations (SD) were obtained from 3 replicates.  $p$  values of <0.05 were considered statistically significant according to the Tukey HSD test.

## 3. Results and Discussion

### 3.1. Effect of the Duration and the Concentration of Zinc Sulfate Priming on Quinoa "Q5" Germination under Salt Stress

The results showed that salinity treatment (200 mM) severely affected seed germination in the ICBA-Q5 genotype mainly after 48 h of germination. The concentrations of 1 g/L of ZnSO<sub>4</sub> presented the highest improvement in germination compared to the negative control

(unprimed). We noticed that there was no significant difference between the durations of 6, 8 and 12 h of treatment. Based on these results, we retained the ZnSO<sub>4</sub> concentration of 1 g/L with 8 h as the treatment duration.

3.2. Effect of the Duration and the Concentration of Zinc Sulfate Priming on Quinoa “Q5” Germination under Salt Stress

The results from the second experiment confirmed that, under the priming condition, Puno and ICBA-Q5 presented the highest-rated results on the FGP and the variety Titicaca had the lowest rate. On the other hand, this parameter was significantly improved by the zinc sulfate priming, but only under 300 mM of NaCl for all the genotypes.

3.3. Effect of Priming with Zinc Sulfate on Germination in Three Quinoa Genotypes

The results illustrated in this part of the experiment proved that the applied salinity levels 300 and 400 mM NaCl drastically reduced the FGP in all of the tested varieties, with a variation between them (Figure 1 A). Indeed, under 300 mM NaCl, the variety Puno was the least affected, followed by ICBA-Q5 and then Titicaca. The salinity level of 500 mM NaCl was too high to completely inhibit seed germination in the tested genotypes, especially in ICBA-Q5 and Titicaca. The zinc sulfate priming treatment improved the germination in the three genotypes. This effect was evident mainly for 300 mM NaCl and was more pronounced for the Titicaca variety for which the induced increase in FGP was more than 100%. Under 400 mM NaCl, the positive effect of priming induced increases in FGP of around 100% for all of the tested varieties. The results of Figure 1B showed that salinity stress increased the MGT in all of the tested varieties but the priming treatment with zinc sulfate reduced this parameter compared to the unprimed seeds.

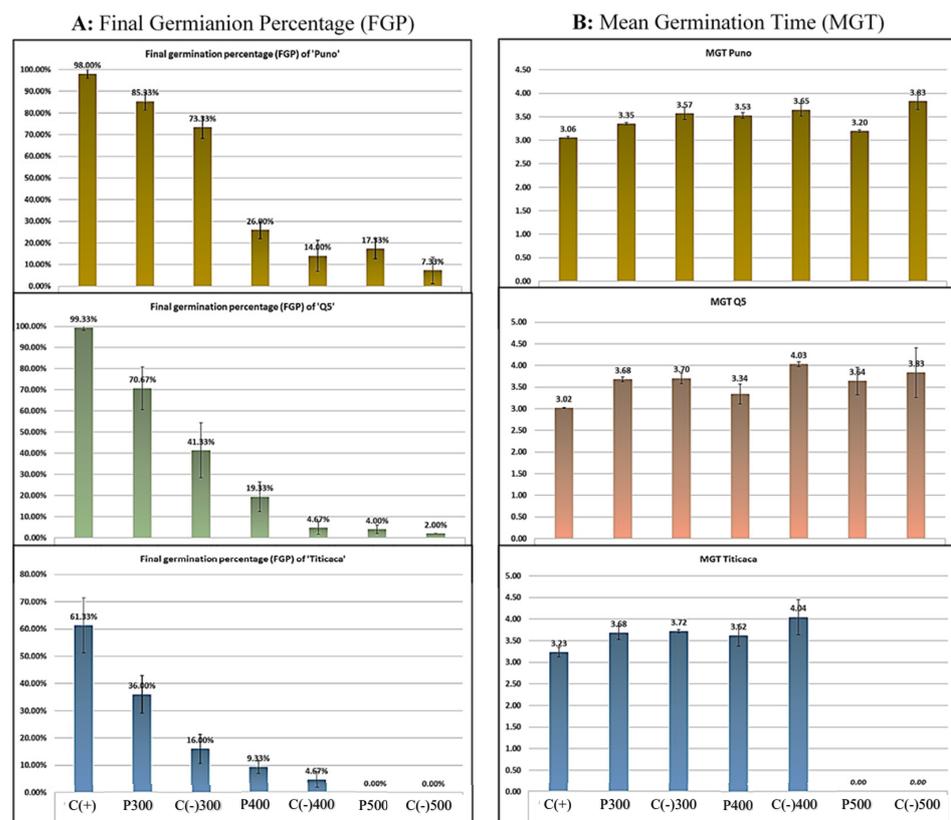


Figure 1. Effect of zinc sulfate priming on the final germination percentage (FGP) (A) and the mean germination time (MGT) (B) in three quinoa varieties, “Puno, Q5 and Titicaca”, positive control “C(+)”

refers to unprimed and non-stressed seeds; negative control “C(-)300; C(-)400; C(-)500” refers to unprimed seeds germinated in 300, 400 and 500 mM NaCl, respectively; “P300; P400; P500” refers to the primed seeds germinated in 300, 400 and 500 mM NaCl, respectively. Results were means  $\pm$  SD of the three replicates.

Under 400 mM NaCl salinity, seed germination was very low in all the tested genotypes and we can state that this NaCl concentration is the salinity threshold for germination for the studied quinoa varieties.

These results are in agreement with those reported by [6], stating that the priming of zinc sulfate had a strong positive effect on the speed and emergence percentage of wheat seeds grown under rainfed conditions. In addition, [8] reported an enhancement of biochemical parameters and seed germination of chickpeas by zinc sulfate priming.

#### 4. Conclusions

These preliminary results allowed us to conclude that the studied quinoa genotypes presented varied seed germination capacities under high salinity levels, as for 300 and 400 mM NaCl, the Puno variety was the most tolerant as compared to ICBA-Q5 and Titicaca. The priming treatment based on soaking seeds for 8 h in 1 g/L solution of zinc sulfate is efficient for improving the germination percentage by more than 100% under salinity levels of 300 and 400 mM NaCl. The concentration of 400 mM NaCl was considered as the germination threshold for the tested quinoa genotypes, especially for ICBA-Q5 and Titicaca.

**Author Contributions:** Conceptualization, C.G. and M.R.B.; methodology, M.R.B. and C.G.; investigation, M.R.B. and S.C.; resources, A.H.; data curation, C.G. and A.Q.; writing—original draft preparation, M.R.B.; writing—review and editing, C.G. and A.H.; supervision, C.G. and A.Q.; project administration, C.G. and A.H.; funding acquisition, C.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by SALAD project in the framework of ERANet -FOSC-056 program.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Reported data were generated by experimental work of this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

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