Stress responses induced by pre-germination treatments and their identification based on seed germination patterns

Respuestas al estrés inducidas por tratamientos pre-germinación y su identificación basada en patrones de germinación de semillas

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ABSTRACT

Tomato is an important vegetable crop in the world and the fruit is widely known as food and as a protector of health. Seed priming improves its germination potential, better seedling establishment, and vigorous growth. Seed priming in water or chemical solutions is a pre-germination treatment that induces mild or stressful stress during the early phases of germination. The primed seed builds a 'priming memory' necessary to configure an "acquired stress response" and upon subsequent stress exposures, they respond more quickly and robustly. The tomato seeds were primed in water (20 h at 20-21 °C) and in 200 mM NaCl and KNO₃ solutions (10 days at 28-29 °C) (stressful condition) to induce stress responses which were characterized in terms of their patterns of germination and velocity of germination, expressed as the time (hours) to 50 per cent germination (T_{50}), when primed seeds were set to germinate in water and wastewater (stressful conditions). Four replicates of 25 seeds on sheets of filter paper in Petri dishes were moistened with 4 mL of distilled water or wastewater and set to germinate (28-29 °C). KNO₃ induced the fastest and strongest stress response. The T_{50} of germination in water (15 h) and wastewater (27 h) and the patterns of germination were different from those induced by the NaCl solution. T_{50} of seeds germinated in water (41 h) and wastewater (39 h) required more time for the seeds primed in the NaCl solution The induced stress responses did not affect the total germination.

Keywords: Germination kinetics, germination speed, induced stress memory, mild stress.

RESUMEN

El jitomate, hortaliza importante en el mundo como fruto ampliamente conocido como alimento y protector de la salud. Mediante el remojo de semillas su potencial de germinación y su establecimiento se mejoran y las plántulas crecimiento vigorosamente. El remojo de semillas en agua o soluciones químicas es un tratamiento previo a la germinación que induce un estrés leve o estresante durante las primeras fases de la germinación. La semilla tratada construye una "memoria" necesaria para configurar una "respuesta de estrés adquirida" y, ante exposiciones de estrés posteriores, responde más rápidamente y contundente. Las semillas se trataron en agua (20 h a 20-21 °C) y en soluciones 200 mM de NaCl y KNO₃ (10 días a 28-29 °C) (condición estresante) para inducir respuestas de estrés que se caracterizaron por sus patrones y velocidad de germinación, expresada como el tiempo (horas) requerido para el 50% de germinación (T₅₀), al germinar las semillas en agua y aguas residuales (condición estresante). Muestras de 25 semillas (4/

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condición experimental) sobre papel filtro en cajas Petri, humedecidas con 4 mL de agua o agua residual y germinadas a $28-29~^{\circ}$ C. KNO₃ indujo la respuesta de estrés más rápida y fuerte. El T_{50} de germinación en agua (15 h) y aguas residuales (27 h) y los patrones de germinación fueron diferentes a los inducidos por la solución de NaCl. El T_{50} de semillas germinadas en agua (41 h) y aguas residuales (39 h) requirió más tiempo para estas semillas. Las respuestas de estrés inducidas no afectaron sensiblemente la germinación total.

Palabras clave: Cinética de germinación, estrés leve, memoria de estrés inducida, velocidad de germinación.

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INTRODUCTION

Tomato crop, cultivated in almost every country of the world is quite important regarding both income and nutrition (Quinet et al., 2019). Tomato is used for the fresh market and tomatoes are grown outdoors and mechanically harvested for the canning industry. The fruit is widely known as food and as a protector of health due to its content and outstanding nutritional value (it is an excellent source of organic acids, essential amino acids, vitamins A and C, minerals such as iron, phosphorus, lycopene, beta-carotene pigments, and dietary fiber) (Shi and Le Maguer, 2010; Kheyrodin, et al., 2017). Tomato has been bred to improve yield, fruit quality, and resistance to biotic and abiotic stresses and the most common strategy widely applied to improve this crop is the traditional plant breeding procedures (Moose and Mumm, 2008). Due to its agronomical and fruit-important features, tomato is becoming a model organism for developmental and stress biology and food science issues (Kimura and Sinha, 2008; Quinet et al., 2019; Liu et al., 2022a). Plants have always coped with changing abiotic conditions. Their subsistence, growth, and productivity have been disturbed by environmental abiotic stresses throughout their approximately 500 million years of evolution, but they have generated and diversified defense response strategies to the different types of environmental stresses (Chen and Soltis, 2020). Seeds and plants have evolved multiple mechanisms to perceive environmental threats and make, in response, suitable adjustments to improve their physiologic and biochemical functions to survive, germinate and grow, known as a "molecular stress memory" (Yuan et al., 2024). The molecular stress memory is used to develop the "acquired stress response" based on strategies that have been developed throughout their evolutionary process (Sani et al., 2013; Hilker et al., 2016). Such response is based on a set of epigenetic adjustments, regulated through signaling pathways and modulated by DNA methylation, histone modification, and alterations of the chromatin structure and regulation of physiological, metabolic, developmental, cellular, and molecular processes in response to future reoccurring environmental stress events (Lamers et al., 2020; Zhang et al., 2022; Zhang et al., 2023). As plants quickly respond forcefully, consequently, they have a better chance of surviving (Hilker et al., 2016). For example, a treatment that causes a non-lethal heat stress condition directs the processing of the primary transcription unit of the mRNA (pre-mRNA), whose products are expressed and act as an acquired stress response, upon a second exposure to heat stress (Mauch-Mani et al., 2017).

Seed priming is a practical and secure approach to improve seed germination potential (Ramírez et al., 2022), better seedling establishment, vigorous growth, and high yields (Adhikari et al., 2022; Granata et al., 2024). This process activates the metabolism that occurs along the phases of germination before the root emergence, followed by drying near the original moisture content. Seed priming in water or chemical solutions is a pre-germination treatment that induces mild or stressful stress during the early phases of germination (Borromeo et al., 2025).

The positive impacts of pre-germination treatments also play a critical role in boosting tolerance to environmental stress conditions. This ultimately results in improved crop quality and yield. Seed priming is a practical but powerful treatment to improve seed germination potential (Ramírez, et al, 2022) and according to several authors (McDonald, 1999; Farooq et al., 2019; Sen and Puthur, 2020) "it consists of soaking the seeds in water or a solution of any agent, followed by drying the seeds, that have started the processes related to germination, but without the appearance of the radicles". Several authors (Powell and Matthews, 1978; Chen and Arora, 2013; Srivastava et al., 2021; Liu et al., 2022: Noble et al., 2023) indicate that this procedure "imparts a moderate abiotic stress to seeds while soaking during the early phases of germination and, depending upon the priming agent, stress effects occur in seeds and finally stimulate stress responses". The primed seed develops more orchestrated the last remaining physiological events for germination to occur and thus is capable of a) greater germination capacity and in a more synchronized manner (Harris and Jones, 1997; Reed et al., 2022; Adhikari

et al., 2022), b) better seedling establishment, c) vigorous growth (Omidi, et al., 2009; Shabbir et al., 2013; Sofo et al., 2014; O'Callaghan, 2016; Liu et al., 2022). Secondly, priming establishes improved abiotic stress responses in seeds, they respond quickly and forcefully and therefore, exhibit greater stress tolerance (Chen and Arora, 2013; Sher et al., 2019; Marthandan et al., 2020; Arun et al., 2022; Louis et al., 2023). Authors (Liu et al., 2022b; Kambona et al., 2023) indicate that "Stress memory responses and seed priming correlate with drought tolerance in plants". Faroog et al., (2019) indicated, that "Seed priming represents a versatile approach for mitigating stress-induced damage in widely cultivated local crop varieties". In the imbibition of water by seeds, fast water uptake occurs in phase I, phase of great metabolic activity in phase II, and uptake of water and germination in phase III (Bewley and Black, 1978; Heydecker and Gibbins, 1978). Water uptake triggers the "germinative metabolism" (e. g. respiration, tranlation of RNAs and synthesis of germination-associated early proteins, mitochondrial, cell membranes and DNA repair, the breakdown of storage reserves, endosperm weakening, etc.) which takes place from the beginning and along phase II of the imbibition process (Forti et al., 2021; Diya et al., 2024). The objective of this work has been to study the impact of mild (seeds exposed to pregermination treatments in water) and severe stress conditions (seeds exposed to pre-germination treatments in salty solutions) on the expression of stress responses. The differentiation of the seed responses was based on the speed of germination (T₅₀) and patterns of germination when seeds were set to germinate in water and under stressful conditions (wastewater). The second objective was to identify the pre-germination treatment that ensures the fastest stress response.

It is considered that seeds acquire molecular stress memory through an initial pre-germination treatment of mild stress. The objective of this research is to investigate the variations in the expression of molecular stress memory when it is induced in seeds by the application of pre-germination stress treatments. After soaking-drying the seeds, determine the acquired stress response by comparing the patterns of germination kinetics and speed of germination as criteria when germinating the seeds in water and under stressful conditions.

MATERIALS AND METHODS

Plant material. Solanum lycopersicum L. (Tomato) seeds variety Río Grande from Hortaflor stored for 6 years (aged seed) and 2 years (unaged seeds), at room temperature. Residual sludge resulting from the treatment and cleaning of wastewater station located in the munic-

ipality of Atlacomulco, State of Mexico. Determination of the weight of absorbed water by 1.5 g of seed soaked in water at 21-22 °C throughout the 54-hour period. Two samples of 1.5 g of seed were placed inside a plastic cloth bag, soaked for 1 sec in the water, shaken vigorously to determine its wet weight at To, and immediately submerged into 1000 mL of water at 21-22 °C. Every hour, the two samples of seed were weighed to determine the weight of water absorbed by them. The results presented were the average of the two samples. Preparation of a wastewater mud sample. 100 grams of sludge were mixed with 100 mL of sterile distilled water and, after shaking very well for 5 min, it was settled for half an hour to recover the supernatant. The supernatant was mixed 1:1 with sterile distilled water and sterilized in an autoclave at 1.02 kg/cm² for 18 min. Pregermination treatments. Seed imbibition in static distilled water. The seed was imbibed for 20 h in 1000 mL of static distilled water at 20-21 °C. Seed imbibition in aerated distilled water. Seed was imbibed for 20 h in 1000 mL of aerated distilled water using a fish tank pump and a Biozon Fragor diffuser stone, at 19-20 °C. Then, all the seed was dried (75 min) in front of a fan. The dried seed was kept in the dark. Seed imbibition in 200 mM of KNO₃ and NaCl solutions was carried out by dispersing the seed on 3 sheets of sterile paper towels, in Petri dishes, moistened with 5 mL of the KNO₃ or NaCl solution. The dishes were placed in a germination chamber (28-29 °C) in the dark for 10 days. Then, the seed was washed with running tap water (30 seconds) and placed in Petri dishes to dry (75 min) in front of a fan. The dry seed was kept in the dark. The control seed did not receive pre-germination treatment.

Seed germination. Samples of 25 seeds (4 per experimental condition) were dispersed on triple sheets of sterile filter paper into Petri dishes, the paper was moistened with 4 mL of sterile distilled water or wastewater, as the case may be. The seeds were placed in a germination chamber (28-29 °C) in the dark. The quantification of the germinated seed was carried out on the indicated days (Coolbear *et al.*, 1984).

Statistical analysis. The experiments were laid out in a completely randomized block design.

RESULTS AND DISCUSSION

Seed vigor is a trait that involves: seed dormancy, high viability, fast and synchronized germination, tolerance to aging, and optimal seedling establishment, particularly under stressful conditions. As seeds age, they progressively lose their vigor condition, and they become increasingly sensitive to priming stress that occurs between the start of priming and radicle emergence

(Bewley et al., 2013). Priming is a transient environmental cue condition, that induces a process to generate adaptive responses expressed usually more rapidly and stronger when exposed to subsequent stress events (Sharma et al., 2022). Since imbibition of seeds in water is a method commonly used to improve their functions (Artola et al., 2003), it was relevant to define the pattern of water absorption by tomato seeds until the moment of germination. The hygroscopic properties of seeds are associated with their seed coat and internal structure and constitution (Miano and Augusto, 2018; Saleh et al., 2018). The water absorption rate can be understood as moisture diffusion, and as the seed absorbs water, increases its moisture content, a rate that decreases as the process progresses. The tomato seeds (1.5 g) after 8, 24, and 54 h of imbibition in water absorbed 36, 55, and 61% respectively of their original dry weight (Figure 1). After 54 h of imbibition, ~1% of the seeds had already germinated. After 54 hours of imbibition, the seed should have carried out phases I and II of the pregermination processes. The degree of hydration of the seed is an important requirement that is combined with time for the seed to reach germination capacity. The critical moisture content has been examined by several researchers (Chachalis et al., 2008; Singh et al., 2013; Asomaning and Sacandé, 2019) and also by studying soil moisture (Hou et al., 2022).

Seeds, after a few minutes of hydration, reestablish respiration (oxygen consumption and CO_2 release) to generate energy to carry out metabolic activity before germination. For this reason, providing O_2 to the imbibition

medium should cause a considerable increase in germination (Al-Ani and Pradet, 1985; Al-Ani et al., 1995; Bewley et al., 2013). Oxygen is also a key signaling factor in the control of seed germination and dormancy (Corbineau, 2022), and with it, the reactivation and establishment of metabolism during seed imbibition, which leads to the production of reduction power and energy (ATP). Liu et al., 2012 demonstrated that oxygen improved the metabolism of seeds, and high oxygen availability in the medium increased the vigor and germination of stored tomato seeds, since the presence of 0.15% H₂O₂ caused the best seed germination. Yasin and Andreasen, 2016 demonstrated that reduced oxygen concentrations can change both the proportion of seeds that germinate and the germination speed (T₅₀). The germination rate of S. lycopersicum seeds decreased if the O₂ concentration was below 10%. Seeds of most species fail to germinate with restriction or lack of oxygen. Tomato seeds germinate 50% in 7 days in water at 25° C with around 5% oxygen (Corbineau, 2022). The sensitivity to lack of oxygen decreases with decreasing temperature, probably because the solubility of oxygen in water increases with decreasing temperature (Smok et al., 1993). Artola et al., (2003, 2003a) demonstrated that, by imbibing the seed in water, it is possible to increase the vigor condition of the Lotus corniculatus L. seed and developed a vigor test based on the reduction of O2 in the germination medium (Artola et al., 2004). Vidal-Lezama et al., (2018) found differences in daily germination due to the effect of O₂ and the average germination speed (T₅₀) was much shorter when the seed was soaked in water with high oxygenation. Based on this

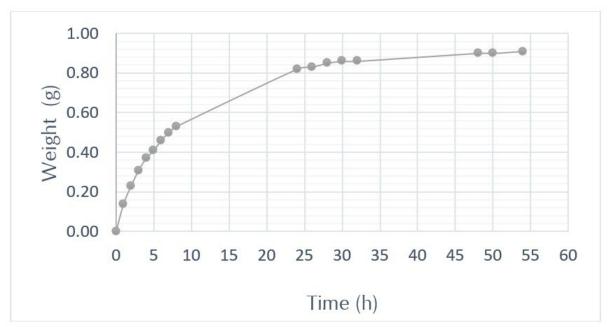


Figure 1. The weight of absorbed water by 1.5 g of seed soaked in water at 21-22 °C throughout 54 hours.

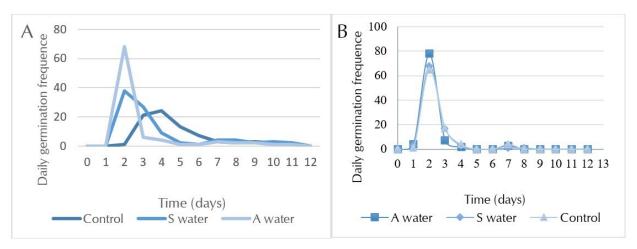


Figure 2. Patterns of germination exhibited by tomato aged seeds (A) and unaged seeds (B) Rio Grande variety, which had previously received a pre-germination treatment of imbibition in water for 20 h at 21-22 °C with aeration (A-water) and in static water without aeration (S-water).

information, it was pertinent to determine how this phenomenon is expressed in aged and unaged seeds of the Rio Grande variety. It can be seen (Figure 2A) that the degree of synchronization exhibited by aged seeds treated with high oxygen content was higher because of the moment at which the maximum frequency of germination was reached at 48 h and the degree of synchronization of the seed keeping in static water was 30% less. Unaged seeds under the three conditions show a high degree of synchrony (Figure 2B). These results have shown that the pattern and degree of synchronization depend on the physiological condition and potentiality of seeds to germinate earlier. The procedure applied in this work to considerably increase the synchronization of the seed was practical, fast, and safe and was only based on the oxygenation of the water. Other methods that have been described to improve seed synchronization are time-consuming and labor-intensive (Heydecker and Beryl, 1978). For example, increases in the synchronization of germination of celery seeds were obtained by treating the seeds with a solution of the salt mixture of c.-10 bar potential at 15 °C for 21 days. This treatment resulted in 50% germination of viable seeds in 1 to 4 days at 20 °C, compared to 13 to 7 days for untreated seeds (Salter and Darby, 1976). Another developed method consists of subjecting a seed population to a complex plurality of hydration and dehydration periods (Berrie and Drennan, 1971).

In the next part of this work, the response of seeds was determined when they were subjected to pregermination treatments during 1, 5, and 10 days of imbibition in 200 mM solutions of KNO₃ or NaCl. After priming treatment, seeds were germinated in water to ana-

lyze their germination patterns. The impact of the duration of imbibition in water on the emergence (Ghassemi-Golezani *et al.*, 2016), the development of the crop (Ghassemi-Golezani *et al.*, 2010) and, in yield (Ghassemi-Golezani *et al.*, 2010a) has been studied. The seed primed in the KNO₃ by 10 days exhibited the fastest germination and the final germination was 89% (Figure 3A) followed by the seed treated 5 days; however, the seed primed 5 days in the NaCl solution germinated at the second day 75.9% and the final germination was 86.6%, while the seed primed for 10 days germinated 64% and the final germination was 81.3% (Figure 3B).

According to several researchers, the intensity and duration of environmental stimuli influence the acquired stress response of the plant, mediated by stress-associated molecules and metabolites, including phytohormones (Hilker et al., 2016) and molecular stress memory remains as a somatic memory for the long term (Ling et al., 2018; Srivastava et al., 2021; Nair et al., 2022).

Patterns of germination of seeds that did not receive pre-germination treatment, which were germinated in mild (water) and stressful (wastewater) conditions.

The seed, that did not receive priming treatment, managed to germinate in water after 4 days, 56 % (Figure 4A), and in wastewater (a stressful condition), only 13 % (Figure 4B).

These results give an idea of the time required for the seed to germinate under stress conditions. The total germination registered on day 10 was similar in both kinds of seeds, in water 75.3 and in wastewater at 72.0 %.

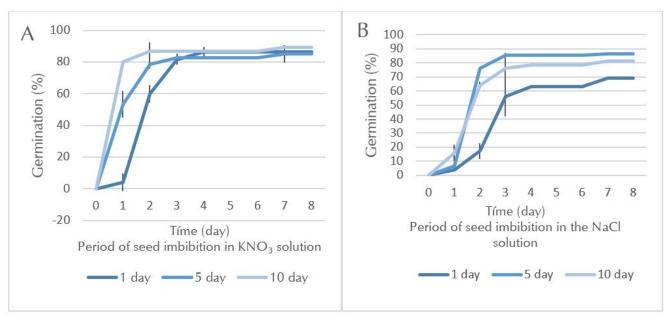


Figure 3. Germination kinetics of seeds exposed to pre-germination treatments in 200 mM KNO $_3$ (A) and 200 mM NaCl solution (B) for 1, 5, and 10 days. Seeds were germinated in distilled water.

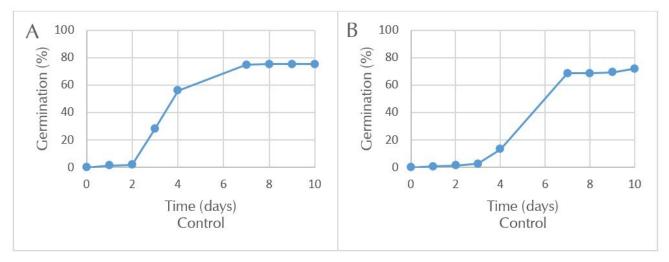


Figure 4. Germination pattern of seeds in water (A) and in wastewater (a severe stressful condition) (B). Seeds did not receive priming treatment.

Patterns of seed germination of seeds that were exposed to a mild pre-germination treatment (imbibition for 20 h in aerated distilled water) which were germinated in mild (water) and stressful (wastewater) conditions.

Seeds that received pre-germination treatment in aerated water on day 4 germinated 80% in water (Figure 5A) and 68% when germinated in the presence of wastewater (Figure 5B). These results demonstrate the effect of pre-germinative treatments on the expression of the induced response, according to the germination patterns of the seeds. The purpose was to test whether it is

possible to differentiate the expression of the induced stress response in seeds when they germinate in water and under stressful conditions. When comparing the germination patterns of seeds that did not receive priming treatment shown in Figures 4A and 4B and the corresponding patterns of seeds that were exposed to a mild pre-germination treatment shown in Figures 5A and 5B, great differences resulted, which demonstrate the positive effect of the pre-germinative treatment. It also positively affected total germination. Germination of seeds under mild and stressful conditions was 86% and 80%, respectively, which is higher compared to seeds that did

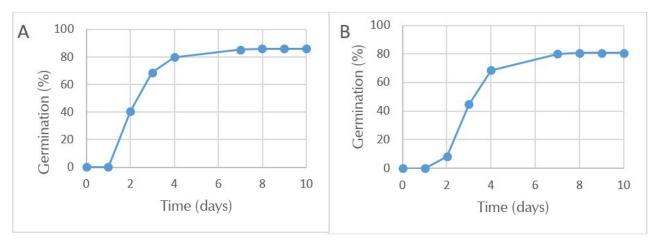


Figure 5. Germination patterns in water (A) and in wastewater (a stressful condition) (B) of seeds that had previously been subjected to pre-germination treatment in aerated water.

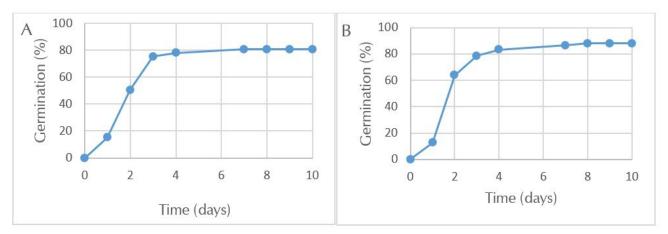


Figure 6. Seed germination pattern in water (mild stress condition) (A) and in wastewater (stressful condition) (B). The seeds had been previously exposed to a stressful pre-germinative treatment in 200 mM NaCl solution.

not receive pre-germination treatment. It is well-studied that priming seeds in water improves germination under stress (Khalequzzaman et al., 2023).

Patterns of seed germination of seeds that were exposed to a pre-germinative treatment in NaCl 200 mM solution which were germinated in mild (water) and stressful (wastewater) conditions.

Seeds primed in the 200 mM solution of NaCl germinated much faster (Figures 6A and 6B) than seeds primed in water. In 3 days, these seeds germinated in water 75 % and under stressful conditions 78 %. The induced stress response was expressed similarly by seeds germinated under both conditions.

Patterns of seed germination of seeds that were exposed to a pre-germinative treatment in KNO₃ 200 mM solu-

tion which were germinated in mild (water) and stressful (wastewater) conditions.

The primed seed in the 200 mM KNO₃ solution (a stressful condition) was able to germinate 70% in water after 24 hours, with a final germination rate of 84.6%. In wastewater (a stressful condition), germination barely reached 37%, and the final germination rate was 80.6%. Again, the most important difference observed was the germination kinetics. The induced stress response expressed by seeds primed with NaCl and KNO₃ solutions differed when seeds were germinated under stressful conditions. This may be due to the chemical differences between the compounds NaCl and KNO₃, since the concentration of both priming solutions was the same. Several researchers have already demonstrated why seeds that have received pre-germination treatments are able to germinate in less time, and they explain that it is

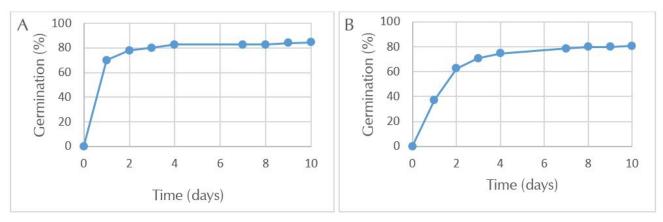


Figure 7. Seed germination pattern in water (mild stress condition) (A) and in wastewater (stressful condition) (B). The seeds had been previously exposed to a stressful pre-germinative treatment in 200 mM KNO₃ solution.

Table 1. T₅₀ and total germination values for seeds exposed to various pre-germination treatments and germinated in different media.

Pre-germination treatment	Germination media	T ₅₀ (hours)	Germination (%)	Standard deviation
Untreated	H ₂ O	80	75.3	4.71
	Wastewater	125	72.0	4.32
Aerated water	H ₂ O	50	86.0	1.63
	Waste water	69	80.7	4.71
NaCl 200 mM solution	H ₂ O	41	80.7	4.71
	Waste water	39	88.0	1.63
KNO ₃ 200 mM solution	H ₂ O	15	84.7	6.80
	Waste water	27	80.7	6.18

due to variations in the expression of the molecular memory of stress. Sani et al., (2013) and Hilker et al., (2016) have shown that the seed before the second stress event, the molecular stress memory was ready to respond more quickly and forcefully. Mauch-Mani et al. (2017) demonstrated that the responses, as well as the level of tolerance expressed for the plants facing up to the second and subsequent stress conditions, differed between primed and unprepared plants. There are variations in the extent of the induced stress response. It is possible to consider that these variations are due also to the degree of the stress condition of the stimulus that induces the molecular stress memory, so the stress responses may be less or more forceful. Sani et al. (2013), also consider that the pre-germination treatment can cause mild or severe stress and they indicate that "Plants can acquire tolerance to lethal levels of stress, by establishing a memory of molecular stress induced by previous exposure to mild or severe transient stress". Bruce et al. (2007) have shown that, after brief exposure to the stress condition, a new cellular state is established, different from the state of the non-exposed plants. Ruttanaruangboworn et al. (2017), investigated the effect of priming seeds with different concentrations of potassium nitrate on rice germination. They demonstrated that priming with 1% KNO₃ showed greater seed germination than priming with 2%. Based on the germination patterns, seeds expressed the fastest induced stress response when germinated in water (Figures 6B and 7B). Therefore, KNO₃ was responsible for the faster germination in seeds set to germinate in water and seeds primed in the solution of NaCl germinated much faster under stressful conditions. The induced stress response induced in seeds by a stressful priming treatment is stronger than that induced by a mild stress priming treatment. Higher germination was observed in treated seeds than in control seeds (Table 1).

It is proposed in this work to evaluate the stress response exhibited by seeds in terms of the rapidity of their expression. According to the data shown in Table 1, there are differences in the results of the T_{50} , between the primed seed in water and germinated in stressful conditions (wastewater), the time to 50% germination was 69 h. When seeds are primed under stress conditions (in NaCl or KNO3 solutions), these events can be recorded by the seeds and stored as "molecular stress memory," which is necessary to configure stress responses to future environmental challenges, which can be rapid and intense. Therefore, under these conditions, the time to 50% germination in wastewater was 39 and 27 h, when the induced stress response was induced by NaCl and KNO₃ solutions, respectively. In this case, the stress response induced by the NaCl solution caused germination to be faster than that of the KNO₃ solution.

Alivia et al., (2025) indicate the advantages of treating the seed with NaCl and textually indicate that "seed priming with NaCl boosted the antioxidant responses in primed chickpea seedlings to stabilize the PS-II function and facilitates the flow of electrons for PS-II, indispensable for energy generation, thus reducing the need of starch degradation and maintaining better starch-sugar equilibrium in primed seedlings".

The benefits of treated seeds extend beyond the germination stage, since they generally generate plants with optimal establishment in the field, with greater vigor and growth. (Omidi et al., 2009; Shabbir et al., 2013; Sofo et al., 2014; O'Callaghan, 2016; Fu et al., 2024; Hasanović et al., 2025; Habibi et al., 2025).

CONCLUSION

In conclusion, germination patterns and T_{50} are informative indicators of induced stress responses. Both NaCl and KNO₃ priming induced responses, with KNO₃ generally producing the fastest kinetics. Germination of primed seeds was faster and more uniform than that of unprimed seeds, without compromising total germination.

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