

Drought stress simulation via mannitol: effects on germination of super strain-B F1 tomato seeds

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ABSTRACT

Drought stress is regarded as the primary limiting factor for different crops within agricultural systems located in arid and semi-arid areas. Thus, choosing suitable plant seeds and assessing their growth under drought stress serves as an effective approach to manage these conditions and yield optimal crops. The tomato plant is an essential annual crop that is adversely affected by drought stress, and its germination stage is notably susceptible. The selection of commercial tomato seeds for economic production is crucial. In this regard, this research focused on analyzing the germination parameters influenced by low water potential induced by mannitol, which simulates drought stress, and the growth of the tomato seed Super Strain-B (F1). This research was conducted at room temperature, where seed germination occurred in Petri dishes with varying concentrations of the osmoticum (0, -3, -6, -9, and -12 bars of drought induced by mannitol). Our findings indicate that drought stress by mannitol significantly decreased germination. The findings indicated that in treatments with -9 and -12 bar potential, the germination parameters of seeds, like germination percentage and rate, mean daily germination, hypocotyl and epicotyl length, and fresh and dry weight of epicotyl and hypocotyl, were significantly lower, with some treatments showing zero amount in comparison to others. In contrast, the control treatment exhibited faster germination parameters, so that complete seed germination occurred by the ninth day. The findings of this study have important implications for helping tomato production and sustainable land management, especially in regions with limited water resources.

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1. Introduction

Global demographic expansion, coupled with diminishing freshwater availability and progressive agricultural land degradation, has necessitated comprehensive investigations into plant stress adaptation mechanisms under suboptimal environmental conditions. Water scarcity constitutes a primary abiotic constraint on crop production globally, with particularly severe impacts in moisture-deficient regions characterized by arid and semi-arid climates (Dietz et al., 2021). Drought stress significantly impairs multiple physiological processes in plants, including reduced seed germination rates, delayed seedling establishment, and compromised photosynthetic efficiency.

Concurrently, water deficit conditions promote reactive oxygen species (ROS) accumulation, leading to oxidative damage, cellular dysfunction, and membrane lipid peroxidation (Rai-Kalal et al., 2021; Hernández-Figueroa et al., 2024; Hossain et al., 2024). These drought-induced physiological and biochemical modifications substantially constrain agricultural productivity, exacerbating food security challenges amid escalating climate variability (Parry et al., 2002; Medeiros et al., 2015). including polyethylene glycol, sorbitol, or mannitol (García-Vásquez et al., 2023). Mannitol (C₆H₁₄O₆), a naturally abundant hexahydric alcohol, possesses considerable



commercial value as a low-calorie sweetener in food applications and as an excipient in pharmaceutical formulations (Chen et al., 2020). Furthermore, mannitol occurs naturally in several economically important Rosaceae species, including apples, pears, peaches, and plums (Godswill et al., 2017). In this regard, various studies have been published concerning the simulation and generation of drought stress by mannitol, which affects specific morphological characteristics and active compounds in plants (Litskas et al., 2019; Yang et al., 2020). On the other hand, the researchers recommend that different stages of growth, including emergence, germination, and growth, should be examined individually when evaluating plants for their ability to withstand environmental stresses. Such analyses could promote the development of cultivars with traits like drought and salinity tolerance (Robin et al., 2014). Murillo et al. (2001) indicated that it is beneficial to select for all traits during the germination and initial rooting stages.

Germination represents a critical physiological transition in plant ontogeny, constituting one of the most vulnerable phases in the life cycle. Under abiotic stress conditions, both seed germination and early seedling establishment emerge as particularly critical stages that fundamentally determine subsequent plant viability (Alaoui et al., 2013). It has been reported the seed germination process is regulated by an intricate interplay of genetic, hormonal, and environmental determinants, and among environmental variables, soil moisture availability represents a critical limiting factor. Meanwhile, water deficit during germination causes particularly severe consequences in arid ecosystems (Kaya et al., 2006). Hence, the germination and initial seedling establishment represent particularly stress-vulnerable developmental stages across numerous plant species, with tomato (*Solanum lycopersicum* L.) demonstrating marked sensitivity to abiotic stressors during these critical growth transitions (Cook, 1979).

Tomato (*Solanum lycopersicum* L.) represents a globally significant vegetable crop, classified within the Solanaceae family comprising approximately 2,800 species (Lahoz et al., 2016). Tomatoes possess considerable nutritional merit, characterized by elevated soluble solid content and strong consumer demand, ensuring favorable economic viability for producers (Maia et al., 2019). With global

production exceeding 182 million metric tons annually, tomato (*Solanum lycopersicum* L.) ranks as the world's second most consumed vegetable crop after potato (*Solanum tuberosum* L.) (Quinet et al., 2019). In this regard, tomato seed physiological quality, quantitatively assessed through germination parameters (total germination percentage, velocity index, and uniformity), significantly influences tomato production efficiency. Optimal seed performance requires not only maximal germination potential but also rapid emergence and homogeneous seedling development, representing critical determinants of overall seed vigor (Finch-Savage, 2016).

While numerous studies have investigated drought stress effects on various plant species under field and greenhouse conditions (Ouzounidou et al., 2016; Kusvuran et al., 2017; Mashilo et al., 2018), and others have employed osmotic agents to simulate water deficit in both in vitro and in vivo systems (Errabii et al., 2006; Gopal et al., 2007; Siaga et al., 2016), no previous research has specifically examined the response of commercial tomato cultivar Super Strain-B F1 to osmotic stress in controlled in vitro culture conditions.

Hence, this work specifically examines tomato (*Solanum lycopersicum* L. cv. Super Strain-B) F1 seed germination under controlled laboratory conditions. The study utilizes mannitol as an external osmotic agent, with the primary goal of studying drought stress effects on both germination parameters and early seedling development by complete monitoring of germination-related indicators. This study will enhance the appropriate response and selection of optimal seeds to drought conditions and agricultural sustainability.

2. Material and methods

This study employed seeds of the commercial tomato hybrid 'Super Strain-B' (*Solanum lycopersicum* L.) to evaluate drought stress responses during germination and early seedling establishment under controlled laboratory conditions. The experimental treatments consisted of a control (distilled water) and mannitol solutions with osmotic potentials of -3, -6, -9, and -12 bar. Each treatment was replicated four times, using the 20 Petri dishes in total for the experiment. The experiment began by lining each Petri dish with a filter paper, followed by the uniform

distribution of 50 tomato seeds on the paper in each dish. Next, varying concentrations of mannitol solutions were prepared, and 7 mL of each solution was added to the corresponding Petri dishes. The dishes were then placed under controlled laboratory conditions to monitor seed germination. Germination was defined as the emergence of a radicle reaching 2 mm in length, with daily counts conducted to record the number of germinated seeds. To maintain consistent moisture levels, an equivalent volume of the respective solution was added to each Petri dish whenever the existing solution approached complete evaporation. Fig. 1. displays tomato seeds subjected to mannitol treatment within a Petri dish.

The completely randomized design with three replications was arranged, and the differences in the germination indices were subjected to an analysis of variance (ANOVA) using Statistix 8 (Tallahassee, FL, USA), and the comparison of means was tested for significance using the least significant difference (LSD) test at a 0.05 level of probability.



Fig. 1. Initial arrangement of seeds in Petri dishes and treatment application.

3. Results and discussion

The ANOVA analysis indicated that the osmotic stress induced by mannitol had a significant impact on the germination and morphological characteristics of the tomato seeds (cv. Super Strain-B), except for hypocotyl length, at a significance level of $p \leq 0.01$ (Table 1). Hypocotyl length of tomato seeds after germination was significantly

influenced by mannitol treatments ($p \leq 0.05$), as shown in Table 1.

Results from mean comparison analyses showed that at 0% drought, strain B seeds achieved the highest germination and morphological traits, in contrast to the lowest values of these parameters observed under -9 and -12 bar drought conditions (Figs. 2-8). A significant decline in tomato seed germination percentage and rate was observed as water potential decreased from control conditions to -9 and -12 bar, indicating severe inhibition under osmotic stress (Figs. 2 and 3). Fig. 3 indicates that the control treatment had notably higher germination rates (93.3%) than any of the osmotic stress treatments. Additionally, the -3-bar treatment recorded the highest rate of this parameter (91.3%) within the stress conditions. A consistent pattern emerged where decreasing osmotic potential correlated with slower germination percentage, rates, and mean daily germination (MDG), with optimal amounts of these parameters occurring in the control treatment (Figs. 2-4). The results indicated that tomato seeds maintained normal germination capacity at water potentials as low as -3 bar, showing no statistically significant difference from the control. However, beyond this threshold, osmotic constraints prevented adequate water uptake, significantly decreasing germination parameters, including percentage, rate, and MDG (Figs. 2-4). Fig. 4 revealed a significant negative correlation between osmotic potential and MDG, where the control and -3 bar treatments exhibited significantly higher germination rates compared to other osmotic stress levels (Fig. 4). These findings align with previous research demonstrating that drought stress significantly impairs both germination parameters and seedling development in tomato plants (Javadi et al., 2018). Also, our findings corroborate the research conducted by Prado et al. (2000) on quinoa (*Chenopodium quinoa* Willd), which demonstrated that the decrease in germination rate is attributed to an osmotic stress mechanism that arises under these conditions, thereby serving as an adaptation strategy to environmental limitations. It has been observed that drought stress not only diminishes the germination rate but also prolongs the germination process by impeding its speed. This postponement may stem from changes in the enzymes and hormones present within the seed.

Additionally, it could be attributed to issues with seed hydration due to elevated osmotic potential, which leads to a specific inhibition of the mechanisms responsible for the emergence

of the radicle from the integuments, thereby resulting in a delay in seed germination (Daroui, 2012).

Table 1. The ANOVA analysis of the effects of mannitol on germination and morphological traits in tomato seeds.

Source	DF	Germination percentage	Germination rate	Mean daily germination	Hypocotyl length	Epicotyl length	FW	DW
Treatments	4	7090.70**	36.60**	82.72**	14.400*	12.84**	182.40**	41.85**
Error	10	14.82	1.95	2.45	1.34	0.64	3.00	0.45
Total	14							
CV		9.79	18.32	16.40	17.29	16.69	19.24	18.85

Note: ** and * indicate significance at the 1% and 5% probability levels. FW stands for Fresh Weight, and DW stands for dry weight.

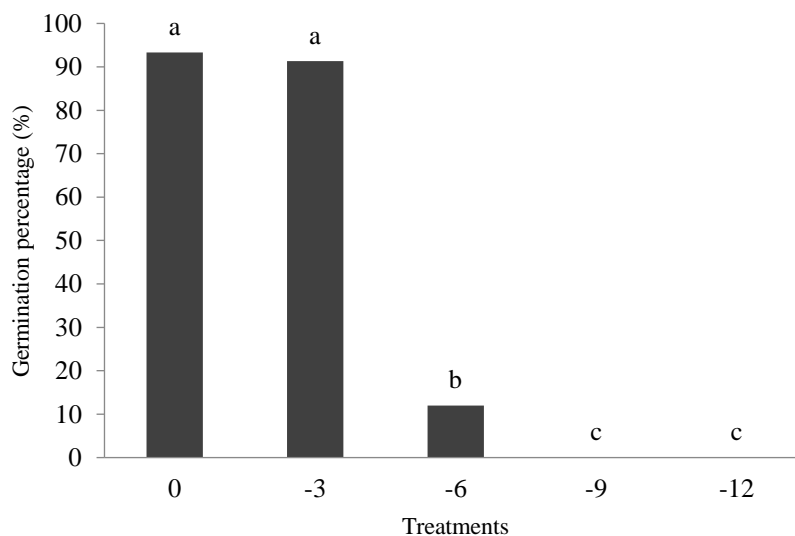


Fig. 2. Germination percentage of tomato seeds.

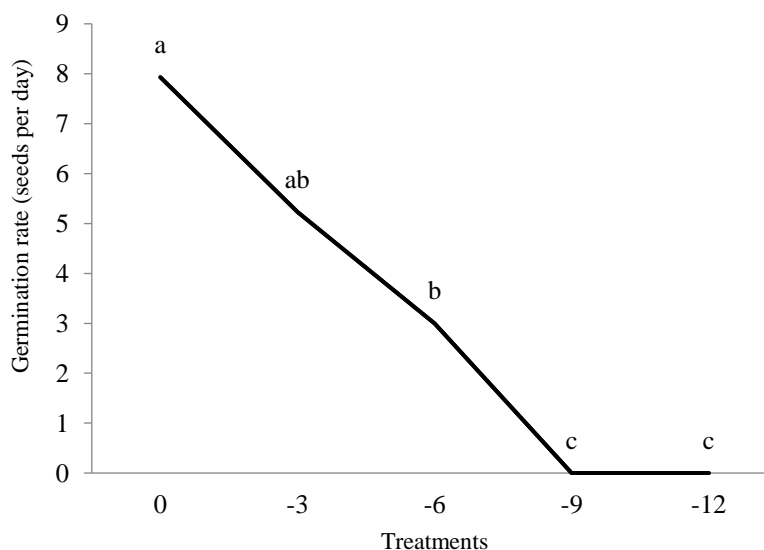


Fig. 3. Germination rate of tomato seeds.

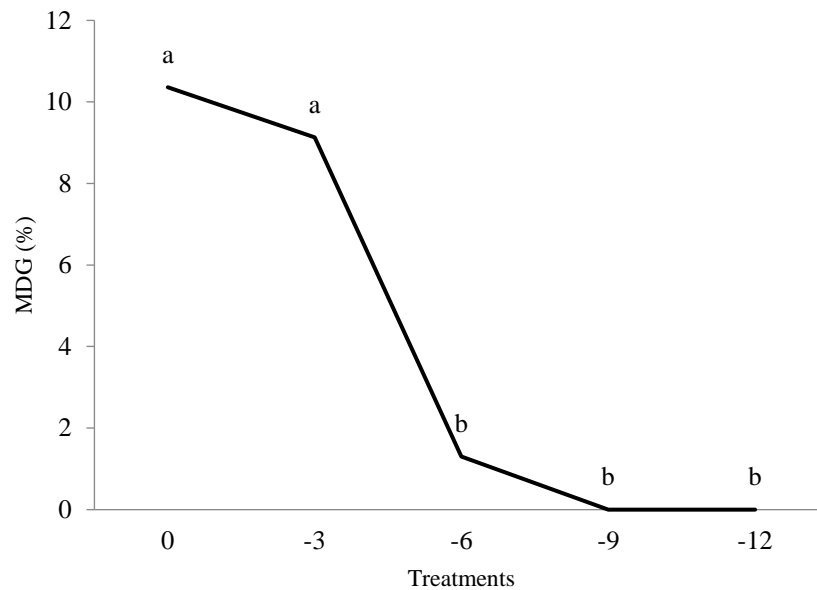


Fig. 4. Mean daily germination (MDG) of tomato seed.

Fig. 5 revealed that hypocotyl elongation was significantly influenced by water potential, with maximal length observed in the control group and minimal growth in the -9 and -12 bar treatments. Notably, hypocotyl development was completely inhibited under severe drought conditions (-9 and -12 bar), and on the other hand, significant differences were not detected among the -3, and -6 bar treatments (Fig. 5). Fig. 6 revealed that epicotyl elongation was significantly reduced under osmotic stress conditions. The control treatment exhibited maximal epicotyl growth, while no statistically significant difference was detected between the -3 and -6 bar treatments. Additionally, the application of -9 and -12 Bar mannitol did not result in any observable growth in epicotyl length (Fig. 6). Our results regarding drought-induced alterations in germination characteristics and seed parameters are consistent with the findings reported by Kavas et al. (2013). Drought stress conditions significantly affect cellular activity in both stem and root meristematic tissues. Consequently, both cell division and elongation processes are impaired, leading to reduced stem and root elongation. Furthermore, water deficit reduces turgor pressure, which is essential for proper cell expansion (Hellal et al., 2018). The current findings align with previous research demonstrating that elevated drought stress consistently reduces germination rates shoot

and root elongation of plants (Feizi et al., 2012). Researchers have indicated that different germination parameters were influenced by the application of mannitol, and the response of seeds varied based on the levels of drought stress and the germination rate, which diminished as the concentration of mannitol increased (Bousba et al., 2021). The parameters under investigation have previously indicated that mannitol, at high doses, exerts an inhibitory effect on the germination of the studied wheat variety seeds (Bousba et al., 2021). This inhibition may be osmotic and/or toxic. To the extent that it is osmotic, we should anticipate that germination will resume once this constraint is removed. On the other hand, if the phenomenon of ion toxicity occurs, we can anticipate the cessation of germination (Hajlaoui et al., 2007). The results showed that fresh weight (FW) and dry weight (DW) of the epicotyl and hypocotyl of tomato seed (cv. Super Strain-B) were reduced significantly with increasing drought stress (Figs. 7 and 8). The highest FW and DW were observed in the control treatment, while the -9 and -12 bar treatments produced the minimum values. Osmotic stress induced by osmotic agents decreased the epicotyl and hypocotyl FW and DW of the tomato compared to the control (Figs. 7 and 8). No significant differences were found between FW and DW of epicotyl and hypocotyl of tomato seed under -9 and -12 bar of mannitol.

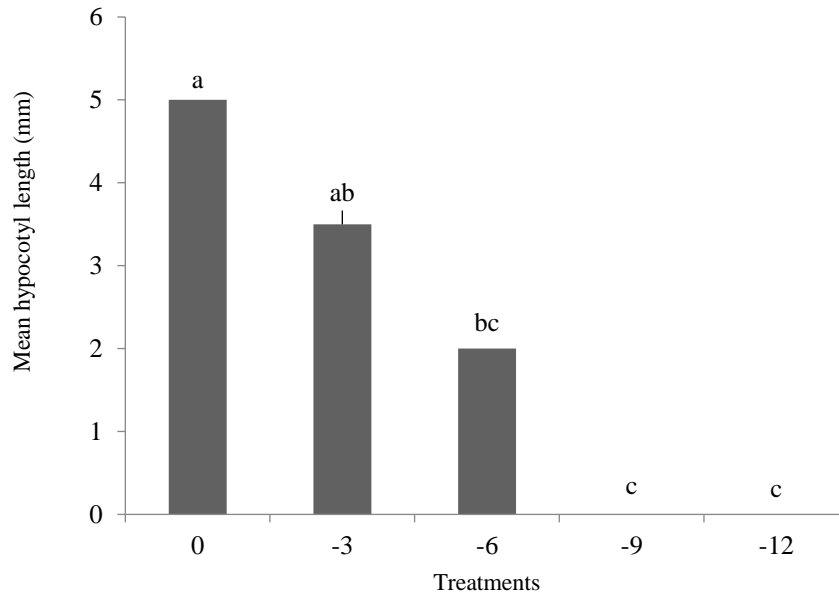


Fig. 5. Mean seed hypocotyl length after tomato seed germination.

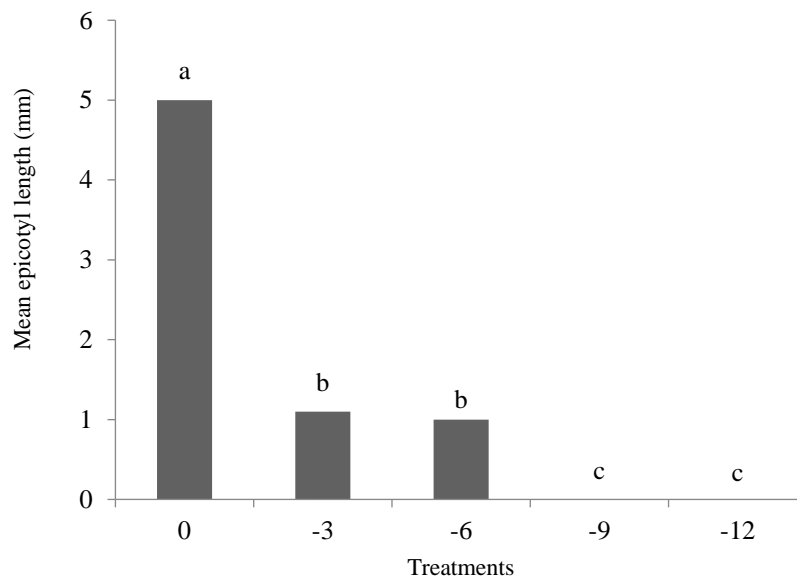


Fig. 6. Mean seed epicotyl length after tomato seed germination.

The observed reduction in germination parameters and seedling growth likely results from impaired water uptake, which slows essential metabolic processes during germination. Our findings align with previous studies demonstrating that mannitol-induced water stress significantly inhibits both fresh and dry weight accumulation during early seedling development (Ochoa-Chaparro et al., 2024).

One of the limitations of this study was the lack of necessary facilities to compare this commercial seed with other widely used seeds globally under drought stress. In this regard, it is recommended that the response of germination parameters of other commercial tomato seeds under drought stress be evaluated, like that employed in this study, to provide more comprehensive results.

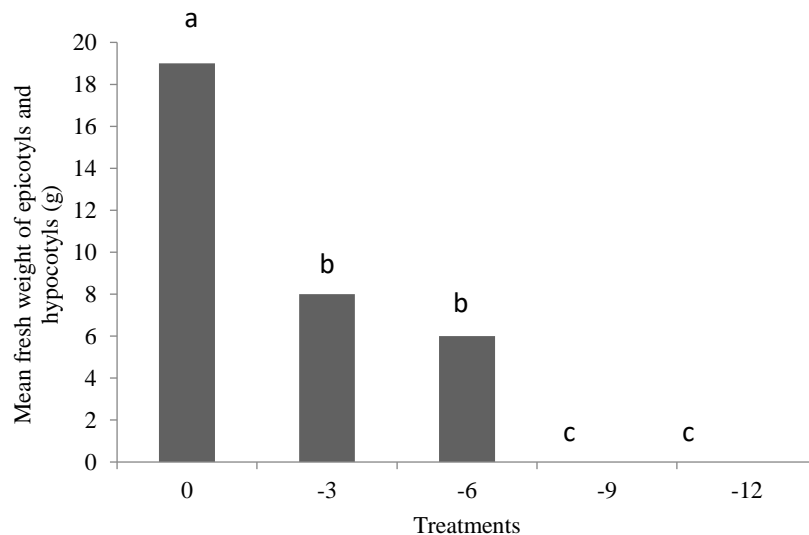


Fig. 7. Mean fresh weight of epicotyls and hypocotyls of tomato seed.

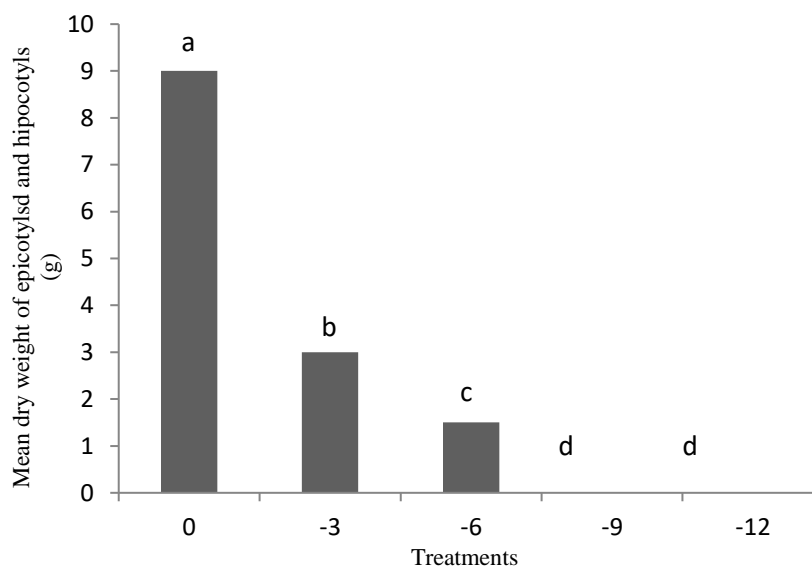


Fig. 8. Mean dry weight of epicotyls and hypocotyls of tomato seed.

4. Conclusion

Due to the limitations of water resources and widespread droughts affecting agricultural production globally, including tomatoes, future studies and research may focus on evaluating the resilience of plants, particularly emphasizing their germination stages under stress conditions. The findings of this research suggest that implementing a test to assess the degree of drought stress tolerance during the germination phases of commercial tomatoes can serve as an approach in a breeding program aimed at identifying potentially tolerant commercial tomato seeds. The study's results, which focused on osmotic stress levels ranging from -3 to -12 bar, were able to highlight

differences in how seeds absorb water and their germination capabilities. Our observations indicated that the different germination parameters were impacted by the osmoticum that was applied, with the responses varying with levels of stress and the germination rate of the variety being tested. In this context, the future perspective may involve evaluating the seeds of various commercial cultivars of plants, such as tomatoes, subjected to different levels of drought stress. The results of this study revealed that tomato seeds (Super Strain-B) need to absorb an adequate amount of water and soften their rigid skin before germination in different drought stress environments. Therefore, it can be inferred that the results of this research may be beneficial for the selection

and application of commercial F1 tomato seeds in drought-prone environments. Finally, the findings of this study have important implications for enhancing tomato production and promoting sustainable land management, particularly in regions with limited water resources.

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