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Onion Seed Germination and Seedling Growth as Affected by PEG-Induced Drought Stress and Ascorbic Acid (Asc) Priming

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ABSTRACT

As agriculture in Iraq faces the challenges of climate change, particularly given the preference for consuming local vegetables, determining the drought tolerance of indigenous varieties and developing strategies to mitigate its impact have become a top research priority. The aim of this study was to mitigate the impact of water potential during the germination stage of a local variety of onion (*Allium cepa* L. var. local white) that is cultivated and consumed widely in the Kurdistan region of Iraq. Ascorbic acid (Asc) with two concentrations (1.5 and 2.5 mM) as a priming compound and polyethylene glycol 8000 (PEG8000) with concentrations (50 and 100 g/L) as a water potential inducer were implemented for different durations (12 and 24 h). Onion seed germination and growth characteristics were recorded. Germination energy at four days (GE4) and germination capacity (GC) were improved when seeds were treated with 1.5 mM Asc for 12 hours. The highest germination rate index (GRI) of 9% and the shortest mean germination time (MGT) were recorded when seeds were treated with 3 mM Asc, while direct sowing had the lowest GRI of 6.5%. The values of radicle and hypocotyl growth were recorded the highest when seeds were primed with 1.5 mM Asc for 12h and exposed to 50 g/L PEG8000. These results indicate the important role of Asc priming under moderate water potential in improving onion seedling growth and resilience. We recommend that farmers in Kurdistan and Iraq adopt Asc priming techniques to enhance drought resistance in onion crops. Future research should focus on optimising Asc concentrations and exploring additional stress-mitigation strategies for other key crops in the region.

تأثير الإجهاد الجفاف الناجم عن بولي إيثيلين جلايكول (PEG) ومعاملة حمض الأسكوربيك (Asc) على إنبات بذور البصل ونمو الشتلات

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الخلاصة

بما أن الزراعة في العراق تواجه تحديات تغير المناخ، لا سيما مع تفضيل استهلاك الخضروات المحلية، أصبح تحديد تحمل الجفاف للأصناف المحلية وتطوير استراتيجيات لتخفيف تأثيره من أولويات البحث الأساسية. كان الهدف من هذه الدراسة هو تخفيف الجهد المائي خلال مراحل الانبات البصل الابيض المحلي (*Allium cepa* L. var. local white) والذي يزرع ويستهلك بكثرة في كردستان. لذلك، حامض الأسكوربيك (Asc) بتركيز 1.5 و2.5 ملي مول ولفترة 12 و24 ساعة كمركب استخدم لمعاملة البذور قبل الزراعة والبولي إيثيلين جلايكول 8000 (PEG8000) بتركيز 50 و100 غرام لكل لتر كمحفز لجهد الماء استخدم في هذه التجربة . صفات انبات البذور ونمو البادرات سجلت تدريجيا. طاقة الإنبات بعد أربعة أيام (GE4) وقدرة الإنبات (GC) تحسنت بعد معاملة البذور ب 1.5 ملي مول Asc ولمدة 12 ساعة. اعلى دليل الانبات GRI بنسبة 9% واقل معدل وقت الانبات MGT سجل بمعاملة البذور ب 3 ملي مول Asc بينما الزراعة المباشرة انتجت اقل GRI 6.5%. عززت معاملة 1.5 ملي مول Asc نمو الجذير والرويشة ، مع ملاحظة أطول طول للجذير (4.2 سم) في البذور المنقوعة لمدة 12 ساعة والمعرضة لـ 50 غرام/لتر PEG8000. تبرز هذه النتائج أهمية تحسين نقع Asc وفهم تأثيرات الجهد المائي لتعزيز نمو شتلات البصل ومقاومتها. نوصي بأن يتبنى المزارعون في كردستان والعراق تقنيات نقع Asc لتعزيز مقاومة الجفاف في محاصيل البصل. ينبغي أن تركز الأبحاث المستقبلية على تحسين تركيزات Asc واستكشاف استراتيجيات إضافية لتخفيف الإجهاد لمحاصيل رئيسية أخرى في المنطقة.

كلمات مفتاحية: *Allium cepa L* البصل ، الجفاف، بولي إيثيلين جلايكول، حمض الأسكوربيك، إنبات البذور

INTRODUCTION

Environmental impacts on crop productivity are a worldwide challenge specially in the areas exposed to drought. In Iraq, insufficient rainfall and reduced water flow in the Tigris and Euphrates rivers has led to prolonged water scarcity, discouraging farmers from cultivation (Hatem *et al.*, 2024). Vegetable crops in Iraq have faced reduced seed germination and reduced growth and development due to drought (Abbas, 2021; Sissakian *et al.*, 2023). Recent reviews highlight that drought adversely affects various aspects of onions (*Allium cepa* L.), including their morphological characteristics, physiological processes, biochemical responses, and genetic parameters (Sansan *et al.*, 2024). To mitigate these effects, onion plants employ adaptive strategies such as developing drought-tolerant varieties, using growth regulators and plant extracts, harnessing plant growth-promoting rhizobacteria, and implementing soil moisture-conserving production and irrigation systems (Gedam *et al.*, 2021; Gökçe *et al.*, 2023).

Seeds are a fundamental component of agriculture, with their quality playing a critical role in growth and seed development. Ensuring the availability of viable and robust seeds during planting is crucial among vegetable crops, onion seeds are noted for their short lifespan, rapidly losing viability post-harvest (Demir *et al.*, 2022). Seed quality is influenced by environmental conditions during growth and seed development (Salih and Kka, 2022; Kamaei *et al.*, 2024). In the Iraq seed industry, certain variables are challenging to control. Additionally, onion seeds exhibit low quality, particularly when subjected to interacting abiotic stress conditions in the field (Ratnarajah and Gnanachelvam, 2021). To achieve rapid and uniform seedling emergence, robust vegetative growth, and high bulb yield, pre-treatment of onion seeds before sowing becomes essential. Ascorbic acid (Asc) (vitamin C) plays a vital role in plant development and abiotic stress tolerance. It has been shown that Asc enhance seedling growth and improve drought tolerance by modulating various physiological processes (Xiao *et al.*, 2021). The seeds of the Red Creole onion variety, widely cultivated in Nigeria, exhibited significantly improved germination percentages when soaked in 12.5% and 25% Asc solutions for 6 and 12 hours (Aluko *et al.*, 2020). Asc used as a pre-sowing seed treatment improves abiotic stress tolerance including wheat (Shah *et al.*, 2019), Linseed (Zrig *et al.*, 2023), rice (Farooq *et al.*, 2006) and maize (Ahmad *et al.*, 2012). Terzaghi and De Tullio (2023) suggests that priming with Asc induces a comprehensive set of coordinated responses, enabling primed seeds to overcome adverse environmental conditions.

In laboratory experiments, polyethylene glycol (PEG) is commonly used to simulate drought stress. By altering the osmotic potential of the nutrient medium, PEG induces drought-like conditions without causing direct cellular or physiological damage (Lei *et al.*, 2021). It was found that treating onion seeds with 10% and 15% PEG 6000 reduced the germination from 92 to 70% , respectively (Muhİe *et al.*, 2020). Similarly, as water potential decreased, final germination rates notably declined, especially when the water potential was below -0.2 MPa (Moradi-Shakoorian *et al.*, 2023). Understanding the impact of PEG-induced drought on seed germination and seedling growth is crucial for developing and identifying drought-tolerant crops.

Studies have explored the interaction between PEG-induced drought stress and Asc metabolism. Asc can mitigate the effects of drought on seed germination and seedling vigor (Terzaghi and De Tullio, 2023). Therefore, this study aimed to assess the effectiveness of Asc under varying water potentials for estimating the drought tolerance of the local white onion variety in Iraq. Germination parameters were also used to predict germination responses to water potential.

MATERIALS AND METHODS

Plant Materials

A 12-day laboratory germination test was conducted using seeds of the white local variety of onion (*Allium cepa* L.) (ISTA, 2021). These seeds were harvested from the experimental farm at the Agricultural Research Center in Ankawa, Erbil, Iraq, during the 2022 season. After harvesting, they were thoroughly dried and stored in a paper package until use.

Treatments

The onion seeds were divided into three lots, each containing 540 seeds. These lots were treated with ascorbic acid (Asc) concentrations of 0, 1.5, and 3 mM (Scharlau, Barcelona, Spain) for 12 and 24 hours, respectively. Additionally, another lot of 270 seeds was directly plated. Table 1 shows the layout of the experimental design. The solutions were prepared using purified and filtered water, and the soaked seeds were stored in darkness at 4°C. After soaking, the seeds were removed from the solution and dried between two layers of towel before plating.Polyethylene glycol (PEG 8000, molecular weight 7000-9000; Promega, Madison, USA) aqueous solutions were prepared to create different water potentials (-0.47 and -1.48 bars). This was achieved by dissolving 50 g and 100 g of PEG in 1L of purified water (Othmani *et al.*, 2021). The control group received purified water (0 bars). The primed and control onion seeds (three replicates of 30 seeds) were placed on two layer of filter paper in 9 cm Petri dishes. 5 ml of each PEG concentration solution (or distilled water for the control) was added to separate Petri dishes to maintain consistent water potentials for each treatment. The Petri dishes were sealed with Parafilm to prevent evaporation. Germination was carried out at 20°C for 12 days according to the principle of ISTA

(2021). The first day of plating count day zero. Seeds were considered germinated when the radicle emerged by at least 2 mm.

Table 1. Layout of the experimental design, rows are highlighted to represent the control treatments where seeds were directly plated, and the last column indicates the number of seeds used for each treatment.

Treatment	Duration (h)	PEG (g/L)	R1	R2	R3	#seeds
No soaking	Direct sowing	0				
		50				270
		100				
0 (H2O)	12h	0				
		50				270
		100				540
	24h	0				
		50				270
		100				
1.5mM AsA	12h	0				
		50				270
		100				540
	24h	0				
		50				270
		100				
3mM AsA	12h	0				
		50				270
		100				540
	24h	0				
		50				270
		100				

Data Collection and Calculation

Germination was registered every 24 h up to 12 days after starting the experiment. The following data were registered:

Germination energy (GE) was calculated for the first four days (GE4) based on the method by Abdelkader *et al*., (2023), six days (GE 6) and germination capacity (GC) after 12 days of germination (Pawłat *et al.*, 2022) days as follows:

$$G = \frac{Germinated\ seeds}{total\ seeds} \times 100$$

Germination rate index (GRI) was expressed in (seed/day) and calculated by the following equation

$$GRI = \sum\left(\frac{Gt}{Dt}\right)$$ ……………… (Abdelkader *et al*., 2023)

Gt refers to the number of germinated seeds on day t, Dt represents time corresponding to Gt in days.

For measuring the rate of germination time-spread per day, mean germination time (MGT) was calculated as follows:

$$MGT = \left[\frac{\sum(D \times N)}{\sum N}\right]$$ …………….. (Abdelkader *et al*., 2023)

Whereas, D is the number of days from the start of the trial and N is the number of seeds germinated on day D.

The fraction of germinated seeds (number of germinated seeds) every day until 12 days (Pawłat *et al.*, 2022).

Vigor index (VI) was also calculated for all treatments as follows:

$$VI = length\ (root + hypocotyl) \times GC$$ ……………… (Abdelkader *et al*., 2023)

The hypocotyl and radicle lengths were measured after 12 days of plating. Photographs were captured using iPhone 13 Pro Max. The image analysis software ImageJ version 1.52n was used to calculate the length of radicle and hypocotyl (cm).
Fresh weight (FW, (mg)); dry matter (DM, (mg)), was determined by drying the fresh seedlings in the drying oven at 65C until constant weight.

Experimental design and statistical analyses

A complete randomize design with three replicates was followed to implement the experiment. The data were analysed using general linear model. The results expressed as mean ± standard error followed Duncan's multiple range test at $p \leq 0.05$ using SPSS v.25.

RESULTS AND DISCUSSION

In general, germination parameters were significantly not affected by Asc and the duration of priming (Figure 1 a, b). Germination energy refers to the percentage of germinated seeds in a seed sample within four and six days in this experiment. Statistically, germination energy was significantly the highest when onion seed were soaked in water (67%), 3mM (65%) and 1.5mM Asc (62%) when compared with no soaking seeds (Figure 1 a). After six days of plating, the GE was become constant within the treatments, however the GE of seeds primed with Asc was more than water and untreated seeds approximately 3%. Recording GE after four days (Abdelkader *et al.*, 2023) and six days (Pawłat *et al.*, 2022) gave similar feature of GE, specifically GE4 improved when onion seeds treated with 0.1% Asc (Mostafa, Leilah and El-Zaidy, 2019). The selective permeability of onion seedcoat (Salanenka and Taylor, 2011) may impact on compound uptake specifically GE4. GE 6 and GC was not affected by using the Asc at two concentration 1.5 and 3mM Asc. In this study, the term "germination percentage" is represented as "germination capacity (GC)." Notably, the highest GC was recorded in non-soaked seeds (93%), while the lowest was observed in seeds soaked in 3mM Asc (91%). Importantly, the statistical analysis did not reveal significant differences between these outcomes. Interestingly, research findings indicate that the effects of Asc are long-lasting. However, despite its impact, a notable proportion of cells in the quiescent center remain unresponsive to Asc, failing to initiate cell division (Innocenti *et al.*, 1990). In addition, the duration of soaking 12h and 24h did not show statistical differences in GE4, GE6 and GC (Figure 1 b), this is in agreement with Aluko *et al*. (2020) who found that soaking onion seeds in Asc for 6h and 12h was not affected on seed germination of Red Creole variety in Nigeria. Therefore, the result of this study demonstrates that priming with Asc has no effect on onion seed germination when compared with water soaking seeds and no soaking seeds. During the initial stages of germination, water potential significantly impacted GE4. In figure 1(c), it is evident that artificial water potential created using PEG 8000 at levels of -0.47 and -1.48 bars resulted in a reduction of GE4 by 10% and 29%, respectively, compared to the control (0). However, the reduction in GE6 and GC due to water potential was not significantly different from the control. This finding aligns with Moradi-Shakoorian *et al.* (2023) who also observed a decrease in onion seed germination with decreasing water potential.

In figure 1(d), the interaction between Asc, soaking duration, and PEG exposure is evident. The highest GE4 was observed when seeds were soaked for 24 hours in water without PEG. Additionally, the highest GE6 and GC occurred when seeds were treated with 1.5 mM Asc for 12 hours and then exposed to a water potential of -0.47 bars. This suggests that Asc at 1.5 mM mitigated the impact of drought stress and balanced the metabolism and radicle emergence of onion seeds. Conversely, the lowest GE4, GE6, and GC were recorded when onion seeds were treated with 1.5mM Asc for 24 hours and exposed to a water potential of -1.48. This leads to the conclusion that a higher concentration of Asc may inhibit signalling specifically from quiescent center cells to the surrounding cells, affecting their division and differentiation (Innocenti et al., 1990).

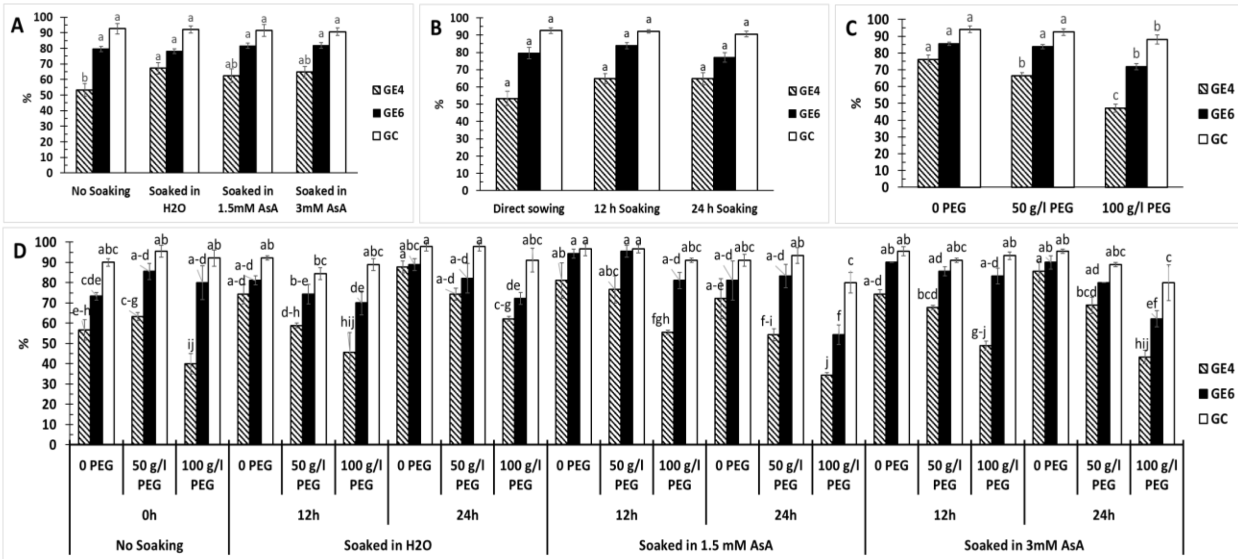

Figure 1. Effects of Asc Priming and Drought Stress on Onion GE4, GE6 and GC. (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). (D) The interaction effect of Asc soaking, duration time, and PEG8000. Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

The 3mM Asc treatment resulted in the highest GRI of 9%, surpassing the GRI of non-soaked onion seeds (Figure 2a) by more than 1.3%. Directly sowing onion seeds without soaking led to the lowest GRI (6.5%), statistically similar to the 1.5 mM Asc soaking treatment. The minimum MGT of 4.1 days occurred with 3 mM Asc, while the maximum MGT of 4.8 days was observed for non-soaked seeds. Soaking seeds for 12 hours yielded the highest GRI (8%) and the shortest MGT (4.1 days) (Figure 2b). These results suggest that higher Asc concentrations may impact seed coat imbibition, permeability, and osmotic pressure. Additionally, seed weight, hardness, and contact surface play significant roles in seed imbibition, as demonstrated in previous studies (Pompelli, *et al.*, 2023). Artificial drought induced by PEG8000 at concentrations of 100 g/L and 50 g/L resulted in a reduction of the GRI by 3% and 1.4%, respectively, compared to the control (0 PEG). Similarly, the mean germination time (MGT) was shortened by 1.3 days and 0.8 days when comparing PEG8000 (100 g/L and 50 g/L) with the control (0 PEG) (Figure 2 c).

Soaking onion seeds in purified water for 24 hours and plating them without PEG resulted in the highest GRI of 11.5% and the shortest MGT of 3.2 days. Conversely, the lowest GRI (5%) and the longest MGT (5.8 days) were recorded when onion seeds were soaked in 1.5 mM Asc for 24 hours and exposed to 100 g/L PEG8000 (Figure 2d). Interestingly, this condition did not statistically differ from both the no-soaking control and the 100 g/L PEG8000 treatment. The presence of 1.5 mM Asc may alter cellular composition, inducing an increase in total sugar and a decrease in protein content. Asc, known for its antioxidant properties, likely influences seed metabolism and germination(Mostafa, Leilah and El-Zaidy, 2019). Proper hydration during seed imbibition is critical, Kamaei *et al.* (2024) demonstrated that storage conditions affect onion seed germination. Specifically, reduced protein concentration and increased levels of glucose, fructose, total sugars, and electrolyte leakage lead to decreased germination percentage and rate.

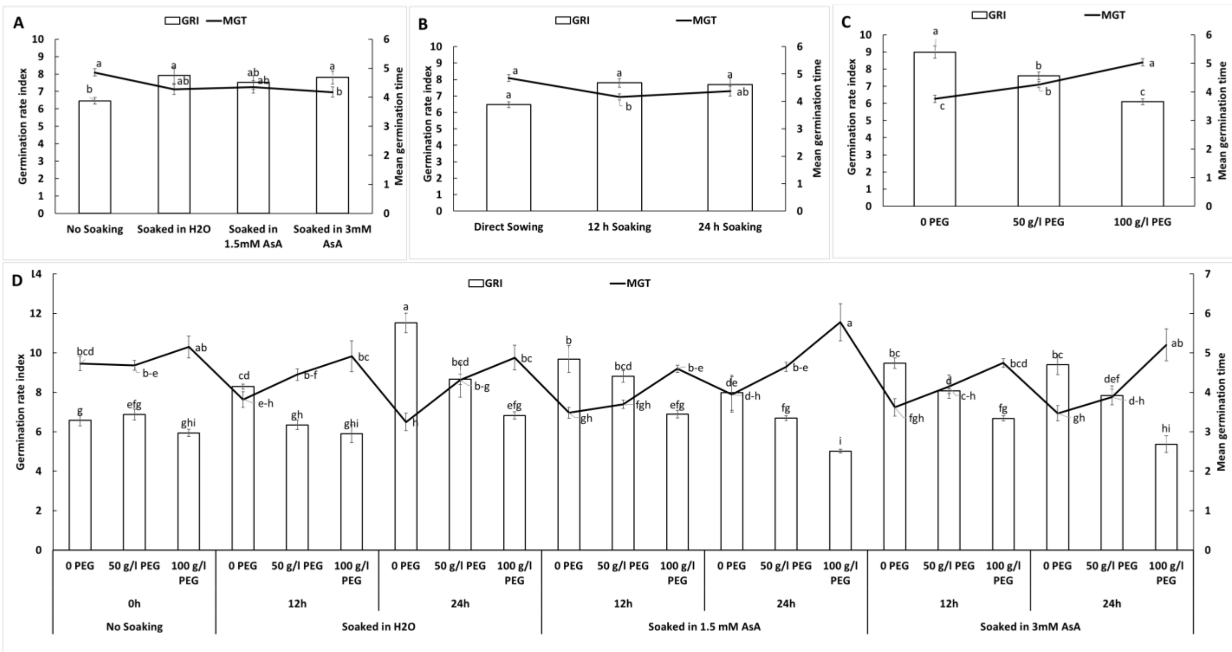

Figure 2. Effects of Asc Priming and Drought Stress on Onion Germination rate index (GRI) and Mean germination time (MGT). (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). (D) The interaction effect of Asc soaking, duration time, and PEG8000. Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

Onion seeds of the local white variety initiate germination after 48 hours of plating, particularly when seeds are primed (Figure 3). However, according to Pawłat *et al.* (2022) Wolska seeds begin germination after 24 hours of plating. Soaking seeds in purified water, 3 mM, and 1.5 mM Asc resulted in the highest fraction of germination (13.5%, 11.6%, and 7.8%, respectively) after 48 hours of plating. These results were statistically higher compared to direct seed plating (0.4%) (Figure 3a). Surprisingly, the same treatments led to opposite outcomes after 12 days of plating. The highest seed germination was recorded in seeds with direct plating (92.6%), while the lowest germination occurred when onion seeds were soaked in 3 mM Asc (90.7%). However, statistically, the values were not significantly different. Figure 3 (a) shows that the fraction of germination for directly plated seeds remains low until five days, after which it remains statistically constant when compared with water- and Asc-primed seeds. This suggests that onion seeds require at least five days to complete their imbibition initiation. The fraction of germination in onion seeds is also affected by the duration of soaking. In this study, the optimal soaking duration for onion seeds was found to be 12 hours (Figure 3b). However, the value varies over time. After 48 hours of plating, the germinated seed values reached 11.5% for 24-hour soaking, 10.5% for 12-hour soaking, and 0.4% for direct plating. Conversely, after 12 days of plating, the fraction of germination reached 92.5% for direct 12-hour pre-treatment and 92.2% for direct 24-hour pre-treatment. Overall, the germination of directly plated seeds remains constant after 5 days of plating and gradually increases. Furthermore, PEG8000 reduced the fraction of germination gradually. Increasing water potential (100 g/L PEG8000) resulted in reduced germination compared to 50 g/L PEG8000. The fraction of germination decreased by 1.6% and 6.0% when treated with 50 g/L and 100 g/L PEG, respectively, compared to purified water (control) (Figure 3c).

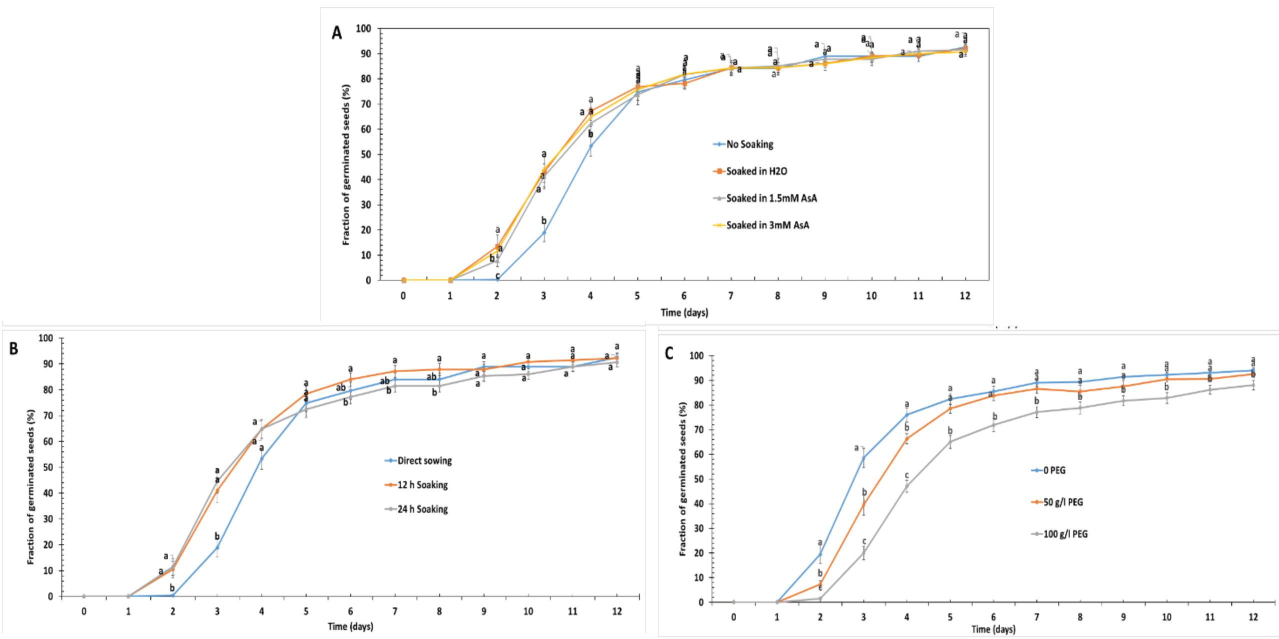

Figure 3. Effects of Asc Priming and Drought Stress on fraction of germinated seeds of Onion (%). (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). The same letter(s) are not statistically significant at $p \leq 0.05$.

The seed vigour index is an important parameter that not only assesses the percentage of viable seeds in a sample but also reflects the ability of those seeds to produce normal seedlings under less-than-optimal or adverse growing conditions, similar to what may occur in the field. In general, the vigour index of onion seeds was statistically not impacted by priming duration with water and Asc when compared with no soaking or direct sowing (Figure 4ab). This finding contradicts the reported results by Mostafa, Leilah and El-Zaidy,(2019) who demonstrated that Asc increased the vigour index (779.67) compared to the control (539.50). However, PEG8000 (100g/L) significantly reduced vigour compared to the control (Figure 4c). The interaction effect graph shows that seeds soaked in water for 24 hours and exposed to 0PEG8000 resulted in the highest vigor index (979), which was statistically not different from seeds primed with 1.5mM Asc for 12 hours and exposed to 50g/L PEG8000 (916), 0PEG (904), and other treatments

(Figure 4d). This outcome indicates that 1.5mM Asc mitigates the impact of water potential caused by PEG at 50g/L. The 24-hour soaking duration with 3mM Asc and exposure during germination to 100g/L PEG resulted in the lowest vigour index, suggesting that the higher concentration of Asc may inhibit the biosynthesis of Asc in the cell and hinder the processes of cell division and elongation.

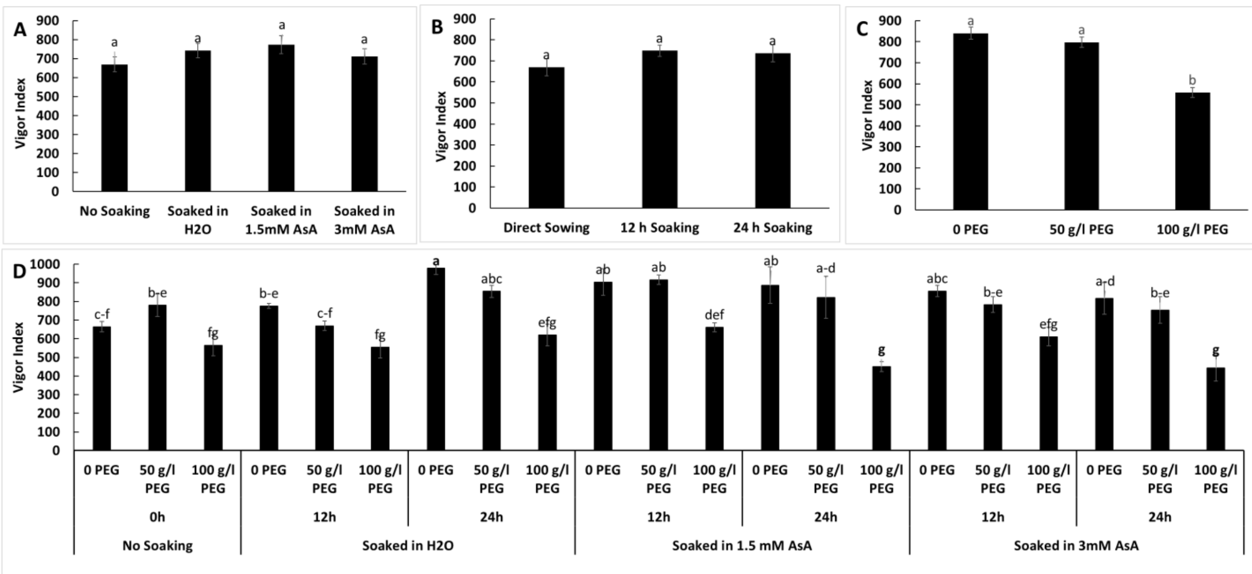

Figure 4. Effects of Asc Priming and Drought Stress on Onion Vigor Index. (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). (D) The interaction effect of Asc soaking, duration time, and PEG8000. Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

Radicle and hypocotyl length are growth characteristics that researchers study intensively to estimate the growth phenomena impacted by different exposures during early seedling development. Specifically, *CsGPA1*-overexpressing transgenic lines, which exhibit enhanced hypocotyl elongation and root growth in cucumber seeds, have been studied (Yan *et al.*, 2018). Additionally, a specific strain of plant growth-promoting bacteria (*ORTB2*) enhanced radicle and hypocotyl length of onion cv. Golden No. 5, resulting in measurements of 1.6 cm and 3.7 cm, respectively, compared to the control (0.7 cm and 1.9 cm) (Samayoa *et al.*, 2020). Furthermore, matriconditioning with synthetic calcium silicate and a growth temperature of 20°C led to enhanced radicle and hypocotyl length compared to the control and growth conditions at 15°C of onion cv. Czerniakowska (Kępczyńska, *et al.*, 2003). In this study, figure 5(a) demonstrates that Asc at 1.5 mM stimulates the growth of radicle and hypocotyl in onion seedlings, resulting in differences of 0.8 cm and 0.3 cm, respectively, compared to no soaking. Although soaking onion seeds for 12 hours and 24 hours did not show statistical differences (Figure 5b), the 12-hour soaking significantly improved radicle growth compared to direct sowing. Similarly, PEG8000 at a concentration of 100 g/L significantly reduced both radicle and hypocotyl length compared to the 0 PEG8000 treatment. The highest radicle length (4.2 cm) was recorded when onion seeds were soaked in 1.5 mM Asc for 12 hours and then exposed to 50 g/L PEG8000, while the lowest value occurred when seeds were soaked in 1.5 mM Asc for 24 hours and then exposed to 100 g/L PEG8000. Hypocotyl length was not significantly affected by the treatments (Figure 5d); however, 3 mM Asc for 24 hours, combined with exposure to 100 g/L PEG, resulted in a statistically significant reduction in hypocotyl length. These results indicate that Asc at low concentrations, rather than 3 mM, stimulates imbibition, metabolism, and radicle emergence in onion seeds. The observed effects on morphological parameters may be attributed to Asc's impact on cell division and elongation. This suggestion aligns with the findings of Kka, Rookes and Cahill (2018) who reported that Asc plays a crucial role in cell division and elongation in *Arabidopsis thaliana*.

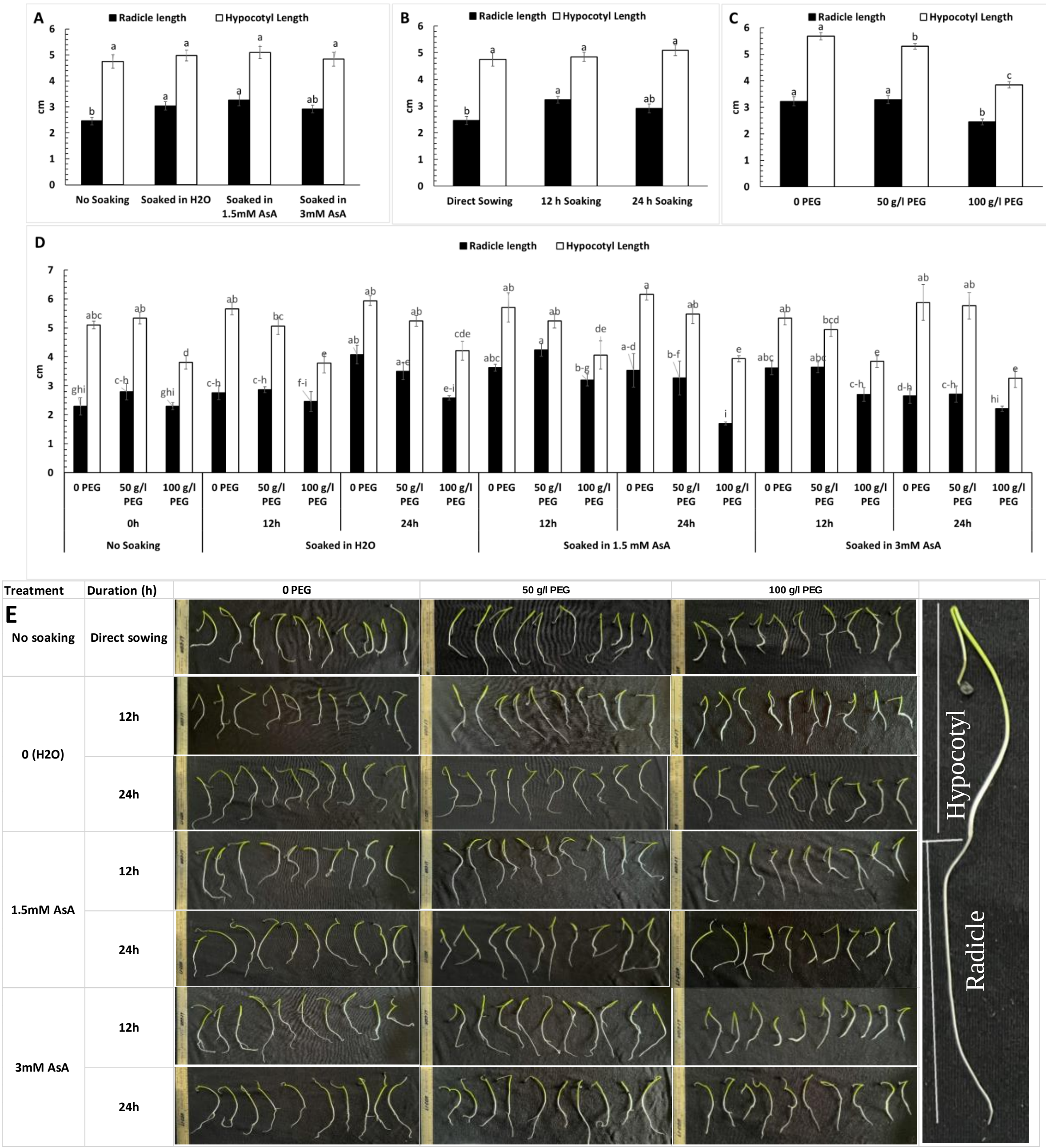

Figure 5. Effects of Asc Priming and Drought Stress on Onion radicle and hypocotyl length (cm). (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). (D) The interaction effect of Asc soaking, duration time, and PEG8000. (E) A representative photograph of 10 seedlings from each treatment after 12 days of plating, on the right side a representative seedling showing the hypocotyl and radicle of onion. Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

The fresh weight of onion seedlings varies slightly with Asc concentration and the duration of primed seeds (Figure 6 a and b). However, exposure to 100g/L PEG8000 significantly reduced the fresh weight compared to the other treatments (Figure 6c). Surprisingly, the dry weight remained statistically constant across all treatments (Figure 6a, b, c, and d).

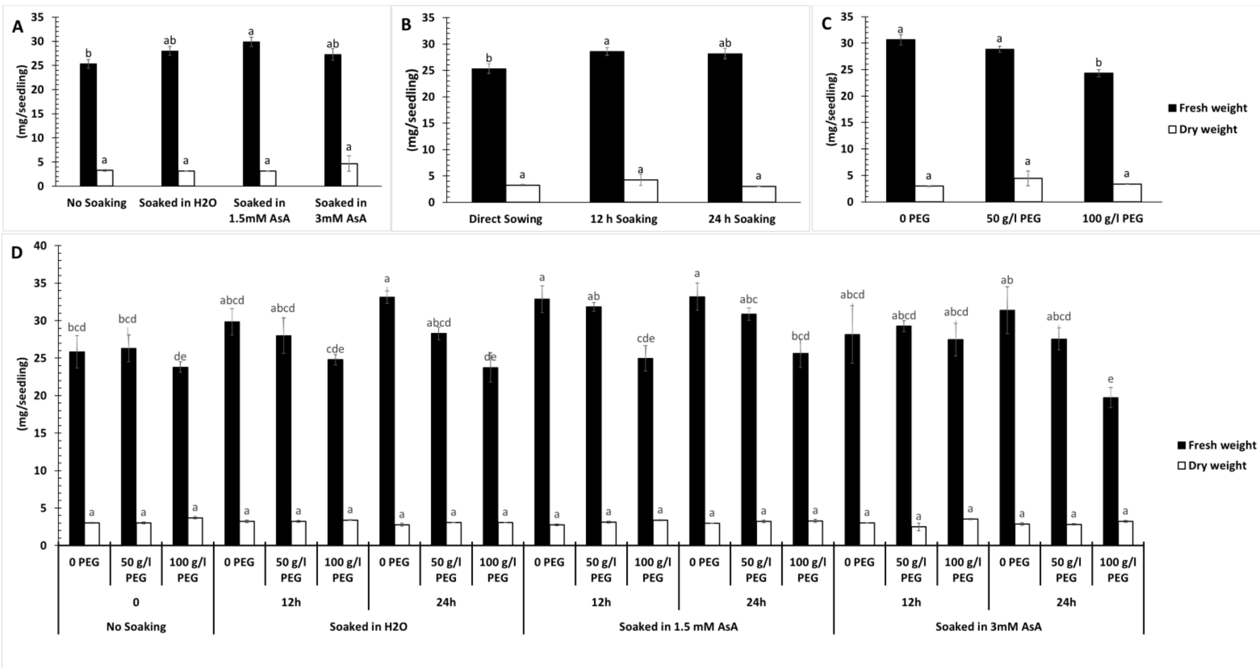

Figure 6. Effects of Asc Priming and Drought Stress on fresh and dry weight of Onion seedling after 12 days of plating (mg seedling). (A) The main effect of soaking seeds in Asc 1.5 and 3mM and compared with seeds soaked in purified water(control) and direct plating. (B) The main effect of soaking duration 12h and 24h and compared with directly plated seeds. (C) The main effect of drought stress (PEG 8000) at two water poetical levels 50g/L and 100 g/L PEG and compared with seeds soaked in purified water (0 PEG). (D) The interaction effect of Asc soaking, duration time, and PEG8000. Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

Briefly, Asc priming had minimal impact on germination characterisation of onion seeds with no statistical differences in GE or GC between Asc treated and untreated seeds (Figure 1a, b). The selective permeability of the onion seed coat (Salanenka and Taylor, 2011) may limit Asc absorption impacting GE4. Similar findings have been reported by Abdelkader *et al*., (2023) and Pawłat *et al*. (2022). Water availability was crucial since PEG8000 induced water potential (-0.47 MPa and -1.48 MPa) significantly reduced GE4, whereas GE6 and GC exhibited no changes (Figure 1c), in agreement with (Moradi-Shakoorian *et al*., 2023). Asc mM was optimised GRI while same treatment reduced the MGT (Figure 2a, b). However, PEG reduce and shortened GRI and MGT respectively, (Figure 2c), demonstrating the role of Asc on osmotic regulation (Mostafa, *et al*., 2019). The results demonstrate that low and moderate Asc concentration promote germination under mild water potential but may hinder development at higher concentrations by disrupting cellular signalling (Kka, Rookes and Cahill, 2018).

CONCLUSION

Asc priming is crucial for enhancing onion seedling resilience under water potential impact. 1.5 mM Asc seed treatment for 12h, under 50 g/L PEG8000 conditions improved germination and growth. While 3mM Asc treatment for 24h under 100 g/L PEG8000 determined the germination. Further studies required to improve priming techniques and implementing the experiment under different environmental conditions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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REFERENCES

Abbas, K. (2021) 'The Effect Of Water Requirements In The Cultivation And Production Of Vegetable Crops (Analytical Study In Agricultural Geography)', *Iraqi Journal of Desert Studies*, pp. 93–115. https://doi.org/10.36531/ijds/21110106.

Abdelkader, M., Voronina, L., Puchkov, M., Shcherbakova, N., Pakina, E., Zargar, M. and Lyashko, M. (2023) 'Seed Priming with Exogenous Amino Acids Improves Germination Rates and Enhances Photosynthetic Pigments of Onion Seedlings (*Allium cepa* L.)', *Horticulturae*, 9(1), p. 80. Available at: https://doi.org/10.3390/horticulturae9010080.

Ahmad, I., Khaliq, T., Ahmad, A., Basra, S.M.A., Hasnain, Z. and Ali, A. (2012) 'Effect of seed priming with ascorbic acid, salicylic acid and hydrogen peroxide on emergence, vigor and antioxidant activities of maize', *African Journal of Biotechnology*, 11(5). Available at: https://doi.org/10.5897/AJB11.2266.

Aluko, M., Ayodele, O.J., Salami, A.E. and Olaleye, O.E. (2020) 'Seed Priming Technique as Innovation to Improve Germination in Onion (*Allium cepa* L.)', *Middle East Journal of Applied Sciences* [Preprint]. Available at: https://doi.org/10.36632/mejas/2020.10.1.2.

Demir, I., Kuzucu, C.O., Ermis, S., Memis, N. and Kadioglu, N. (2022) 'Estimation of Seed Longevity in Onion Seed Lots by a Vigor Test of Radicle Emergence Test in Artificial Ageing Conditions', *Horticulturae,* 8(11), p. 1063. Available at: https://doi.org/10.3390/horticulturae8111063.

Farooq, M., Basra, S.M.A., Tabassum, R. and Afzal, I. (2006) 'Enhancing the Performance of Direct Seeded Fine Rice by Seed Priming', *Plant Production Science,* 9(4), pp. 446–456. Available at: https://doi.org/10.1626/pps.9.446.

Gedam, P.A., Thangasamy, A., Shirsat, D. V., Ghosh, S., Bhagat, K.P., Sogam, O.A., Gupta, A.J., Mahajan, V., Soumia, P.S., Salunkhe, V.N., Khade, Y.P., Gawande, S.J., Hanjagi, P.S., Ramakrishnan, R.S.

and Singh, M. (2021) 'Screening of Onion (*Allium cepa* L.) Genotypes for Drought Tolerance Using Physiological and Yield Based Indices Through Multivariate Analysis', *Frontiers in Plant Science*, 12. Available at: https://doi.org/10.3389/fpls.2021.600371.

Gökçe, A.F., Gökçe, Z.N.Ö., Junaid, M.D. and Chaudhry, U.K. (2023) 'Evaluation of biochemical and molecular response of onion breeding lines to drought and salt stresses', *Scientia Horticulturae*, 311, p. 111802. Available at: https://doi.org/10.1016/j.scienta.2022.111802.

Hatem, I., Alwan, I.A., Ziboon, A.R.T. and Kuriqi, A. (2024) 'Assessment of agricultural drought in Iraq employing Landsat and MODIS imagery', *Open Engineering*, 14(1). Available at: https://doi.org/10.1515/eng-2022-0583.

INNOCENTI, A.M., BITONTI, M.B., ARRIGONI, O. and LISO, R. (1990) 'The size of quiescent centre in roots of *Allium cepa* L. grown with ascorbic acid', *New Phytologist*, 114(3), pp. 507–509. Available at: https://doi.org/10.1111/j.1469-8137.1990.tb00418.x.

ISTA (2021) *International Rules for Seed Testing*. 2021st edn. Bassersdorf, Switzerland: International Seed Testing Association.

Kamaei, R., Kafi, M., Afshari, R.T., Shafaroudi, S.M. and Nabati, J. (2024) 'Physiological and molecular changes of onion (Allium cepa L.) seeds under different aging conditions', *BMC Plant Biology*, 24(1), p. 85. Available at: https://doi.org/10.1186/s12870-024-04773-7.

Kępczyńska, E., Piękna-Grochala, J. and Kępczyński, J. (2003) 'Effects of matriconditioning on onion seed germination, seedling emergence and associated physical and metabolic events', *Plant Growth Regulation*, 41(3), pp. 269–278. Available at: https://doi.org/10.1023/B:GROW.0000007509.94430.eb.

Kka, Noura, Rookes, J. and Cahill, D. (2018) 'The influence of ascorbic acid on root growth and the root apical meristem in Arabidopsis thaliana', *Plant Physiology and Biochemistry*, 129, pp. 323–330. Available at: https://doi.org/10.1016/j.plaphy.2018.05.031.

Lei, C., Bagavathiannan, M., Wang, H., Sharpe, S.M., Meng, W. and Yu, J. (2021) 'Osmopriming with Polyethylene Glycol (PEG) for Abiotic Stress Tolerance in Germinating Crop Seeds: A Review', *Agronomy*, 11(11), p. 2194. Available at: https://doi.org/10.3390/agronomy11112194.

Moradi-Shakoorian, Z., Delshad, M., Diaz-Perez, J.C., Askari-Sarcheshmeh, M.-A., Nambeesan, S. and Mamedi, A. (2023) 'Onion (*Allium cepa* L.) seed germination affected by temperature and water potential: Hydrothermal time model', *Journal of Applied Research on Medicinal and Aromatic Plants*, 35, p. 100495. Available at: https://doi.org/10.1016/j.jarmap.2023.100495.

Mostafa, E.-S., Leilah, A. and El-Zaidy, M. (2019) 'Improving Seed Germination and Seedling Vigor Traits of Onion (*Allium cepa* L.) By using Soaking Pretreatments', *Journal of Plant Production*, 10(12), pp. 1163–1167. Available at: https://doi.org/10.21608/jpp.2019.79700.

Muhİe, S., Özdamar, C., Gökdaş, Z., Njİe, E.S., Memİş, N. and Demİr, I. (2020) 'Effect of solid matrix priming with seaweed extract on germination and seedling performance of onion seeds under abiotic', *Black Sea Journal of Agriculture*, 3(4), pp. 233–238.

Othmani, A., Ayed, S., Chamekh, Z., Slama-Ayed, O., Da Silva, J.A.T., Rezgui, M., Slim-Amara, H. and

Younes, M. Ben (2021) ‘Screening for PEG-induced drought stress tolerance in seedlings of durum wheat (Triticum durum Desf.) cultivars’, *Pakistan Journal of Botany*, 53(3). Available at: https://doi.org/10.30848/PJB2021-3(5).

Pawłat, J., Starek-Wójcicka, A., Kopacki, M., Terebun, P., Kwiatkowski, M., Sujak, A., Pascuzzi, S., Santoro, F. and Andrejko, D. (2022) ‘Germination Energy, Germination Capacity and Microflora of Allium cepa L. Seeds after RF Plasma Conditioning’, *Energies*, 15(20), p. 7687. Available at: https://doi.org/10.3390/en15207687.

Pompelli, M.F., Jarma-Orozco, A. and Rodriguez-Páez, L.A. (2023) ‘Imbibition and Germination of Seeds with Economic and Ecological Interest: Physical and Biochemical Factors Involved’, *Sustainability*, 15(6), p. 5394. Available at: https://doi.org/10.3390/su15065394.

Ratnarajah, V. and Gnanachelvam, N. (2021) ‘Effect of Abiotic Stress on Onion Yield: A Review’, *Advances in Technology*, 1(1). Available at: https://doi.org/10.31357/ait.v1i1.4876.

Salanenka, Y.A. and Taylor, A.G. (2011) ‘Seedcoat Permeability: Uptake and Post-germination Transport of Applied Model Tracer Compounds’, *HortScience*, 46(4), pp. 622–626. Available at: https://doi.org/10.21273/HORTSCI.46.4.622.

Salih, M. and Kka, N. (2022) ‘Evaluation of The Efficiency of Onion (*Allium Cepa* L.) Sets Treated With Ascorbic Acid and Salicylic Acid In Stimulating Growth and Seed Production’, *Mesopotamia Journal of Agriculture*, 50(1), pp. 52–64. Available at: https://doi.org/10.33899/magrj.2022.132858.1163.

Samayoa, B.E., Shen, F.-T., Lai, W.-A. and Chen, W.-C. (2020) ‘Screening and Assessment of Potential Plant Growth-promoting Bacteria Associated with <i>Allium cepa</i> Linn.’, *Microbes and Environments*, 35(2), p. n/a. Available at: https://doi.org/10.1264/jsme2.ME19147.

Sansan, O.C., Ezin, V., Ayenan, M.A.T., Chabi, I.B., Adoukonou-Sagbadja, H., Saïdou, A. and Ahanchede, A. (2024) ‘Onion (Allium cepa L.) and Drought: Current Situation and Perspectives’, *Scientifica*, 2024, pp. 1–12. Available at: https://doi.org/10.1155/2024/6853932.

Shah, T., Latif, S., Khan, H., Munsif, F. and Nie, L. (2019) ‘Ascorbic Acid Priming Enhances Seed Germination and Seedling Growth of Winter Wheat under Low Temperature Due to Late Sowing in Pakistan’, *Agronomy*, 9(11), p. 757. Available at: https://doi.org/10.3390/agronomy9110757.

Sissakian, V.K., Nadhir Al-Ansari, Nasrat Adamo and Jan Laue (2023) ‘The Impact of Water Scarcity on Food Security in Iraq’, *Journal of Civil Engineering and Architecture*, 17(9). Available at: https://doi.org/10.17265/1934-7359/2023.09.003.

Terzaghi, M. and De Tullio, M.C. (2023) ‘Ascorbic Acid in Seeds, Priming and Beyond’, *Seeds*, 2(4), pp. 421–435. Available at: https://doi.org/10.3390/seeds2040032.

Xiao, M., Li, Z., Zhu, L., Wang, J., Zhang, B., Zheng, F., Zhao, B., Zhang, H., Wang, Y. and Zhang, Z. (2021) ‘The Multiple Roles of Ascorbate in the Abiotic Stress Response of Plants: Antioxidant, Cofactor, and Regulator’, *Frontiers in Plant Science*, 12. Available at: https://doi.org/10.3389/fpls.2021.598173.

Yan, Y., Zhang, W., Li, Y., He, C., Gao, L. and Yu, X. (2018) ‘Functions of CsGPA1 on the hypocotyl elongation and root growth of cucumbers', *Scientific Reports*, 8(1), p. 15583. Available at: https://doi.org/10.1038/s41598-018-33782-4.

Zrig, A., Yousif Sidahmed Elsheikh, S., Hamouda, F., Najar, B., A. Alsherif, E., Magdy Korany, S., Hassan, A.H.A. and AbdElgawad, H. (2023) 'Potassium Nitrate and Ascorbic Acid Priming Improved Tissue Chemical Composition and Antioxidant and Antimicrobial Activities of Linseed (*Linum usitatissimum* L.) Sprouts', *ACS Omega*, 8(39), pp. 35975–35987. Available at: https://doi.org/10.1021/acsomega.3c03002.