

Enhancing safflower seedlings' tolerance to osmotic stress through seed priming with glutathione, epibrassinolide, chitosan, and folic acid

Naser Abdiazar, Hossein Zahedi , Younes Sharghi, Seyed A. M. Modarres Sanavy and Akbar Alipour

Address:

Department of Agronomy and Integrated Cropping Research Center, Eslamshahr Branch, Islamic Azad University, Eslamshahr, Iran.

*Corresponding Author: Hossein Zahedi, e-mail: hzahedi2006@gmail.com

Received: 21-02-2024; Accepted: 18-03-2024; Published: 20-04-2024

DOI: [10.21608/EJAR.2024.271848.1518](https://doi.org/10.21608/EJAR.2024.271848.1518)

ABSTRACT

Investigating the effects of priming methods on seedling physiology under osmotic stress conditions is important to understand how priming enhances osmotic stress tolerance in safflower. The mechanisms by which priming achieves this at the physiological level, such as changes in osmoprotectants, antioxidants, and membrane damage, are not well understood. This research aims to fill these knowledge gaps and provide insights that can be used to optimize priming treatments and improve safflower productivity in drought-prone areas. Seeds were treated with priming including distilled water, folic acid, glutathione, epibrassinolide and chitosan and then subjected to osmotic stresses including no osmotic stress, mild osmotic stress (4 bar) and severe osmotic stress (8 bar) for germination and seedling growth. Severe osmotic stress increased soluble leaf proteins, this increase was observed in all priming treatments except distilled water. Severe osmotic stress increased soluble leaf proteins by an average of 35% in folic acid, chitosan, and epibrassinolide treatments. Chitosan treatment exhibited 172% and 188% more proline under mild and severe stress, respectively, compared to the control. Folic acid treatment showed a 207% increase in catalase under severe stress. Epibrassinolide and glutathione treatments had 201% more superoxide dismutase (SOD) under non-stress conditions. Distilled water treatment had 48.6% more soluble sugars under non-stress, while glutathione and epibrassinolide treatments had 14.5% more under mild stress. Control treatment had the highest malondialdehyde (MDA) amounts under mild and severe stress. Chitosan treatment exhibited a 25% increase in seedling length under severe stress compared to the control. Under non-stress conditions, chitosan treatment had slightly lower seedling length compared to control, while glutathione and epibrassinolide treatments showed the highest lengths. Under mild stress, folic acid, chitosan, and epibrassinolide treatments were superior to control. Under severe stress, distilled water showed a 20% increase, while chitosan treatment showed a 25% increase in seedling length compared to control. Chitosan treatment improved safflower seedling growth under severe osmotic stress through mechanisms including increased osmoprotectant levels, enhanced antioxidant activity, and improved membrane integrity.

Keywords: Osmotic stress, priming, osmoregulation, antioxidants, cellular energy.

INTRODUCTION

Safflower is an important oilseed crop cultivated for hundreds of years (Gomashe *et al.*, 2021). It is commercially used for oil extraction, pharmaceuticals and food industries. Its cultivation is more common in dry and semi-arid areas (de AlmeidaSilva *et al.*, 2023) but increasing drought periods due to climate changes even threaten its production in these areas. Water deficiency in early growth stages can reduce yield unless proper management is practiced (Akbari *et al.*, 2020). Seed priming refers to the hydration and dehydration of seeds before germination which leads to the acceleration of metabolic processes without germination (Akbari *et al.*, 2020). This method increases the ability of tolerance by synchronizing germination and establishing seedlings under abiotic stresses. Priming increases accumulation of antioxidants and osmolytes for osmoregulation which enhances seedling tolerance to water deficiency (Marthandan *et al.*, 2020).

To take advantage of priming benefits in safflower cultivation under drought conditions, investigating physiological changes after priming and during seedling growth is essential (Marthandan *et al.*, 2020). Using distilled water or materials like glutathione, epibrassinolide, chitosan and folic acid as priming treatments can improve germination under osmotic stress conditions through increasing antioxidant and defensive system activity of seeds, maintaining moisture and cellular health within seeds and preventing cellular damage due to osmotic stress (Aghaee Marthandan *et al.*, 2020; Rahimi *et al.*, 2020; Burdan *et al.*, 2023; Hidangmayum *et al.*, 2023; Saeed *et al.*, 2023).

Priming treatments using materials such as glutathione, epibrassinolide, chitosan, and folic acid have shown promise in enhancing safflower germination and seedling growth under osmotic stress conditions. These materials act as priming agents by influencing various physiological processes within the seeds and seedlings.

One of the key physiological mechanisms behind the positive effects of priming is the activation of antioxidant systems. Osmotic stress can lead to the accumulation of reactive oxygen species (ROS) within plant cells, causing oxidative damage. Priming treatments enhance the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which help scavenge ROS and protect cellular components from oxidative stress (Marthandan *et al.*, 2020; Saed and Mohhamed, 2023).

Additionally, priming treatments promote the accumulation of osmolytes, such as proline and soluble sugars, within the seeds. Osmolytes act as compatible solutes and help maintain cellular osmotic balance, thereby protecting the cells from water loss and maintaining their structural integrity under osmotic stress conditions (Marthandan *et al.*, 2020).

Priming also influences hormone signaling pathways. For example, epibrassinolide, a brassinosteroid hormone, has been shown to improve safflower germination and seedling growth under osmotic stress. Brassinosteroids play a crucial role in regulating plant growth and development, and their application as a priming treatment can enhance cell elongation, promote root growth, and improve overall plant performance under stress conditions (Marthandan *et al.*, 2020; Kashif *et al.*, 2021).

Furthermore, priming treatments can enhance the water status and cellular health of seeds. They help maintain seed moisture content and prevent cellular damage during the priming process, enabling seeds to withstand and recover from water-deficit conditions more efficiently (Saeed *et al.*, 2023).

Overall, priming with materials influences multiple physiological mechanisms in safflower plants, including antioxidant activation, osmolyte accumulation, hormone signaling, and cellular health maintenance. These mechanisms collectively contribute to improved germination, seedling establishment, and tolerance to osmotic stress, ultimately enhancing safflower productivity in drought-prone areas (Burdan *et al.*, 2023).

While priming has shown promise for improving stress tolerance in various crops, limited research exists on its comparative effects in modulating safflower physiology under osmotic stress. The mechanisms by which priming may enhance tolerance at early growth stages also remain unclear. This study aims to address these knowledge gaps by evaluating multiple priming agents for their ability to modulate key stress response mechanisms in safflower seedlings. Specifically, it will investigate priming-induced changes to antioxidants like SOD and CAT that mitigate stress-induced damage. The study will also examine osmoregulatory solutes like proline and soluble sugars that balance cell turgor and provide energy under water deficit. Membrane stability, indicative of cell viability under stress, will further elucidate priming-conferred protection.

Additionally, previous work has predominantly focused on later crop stages with little emphasis on establishing resilience from seed priming during the critical early seedling phase. This study centers stages of osmotic stress imposition to mimic field conditions where seedlings face greatest moisture stress impact. It also compares widely different, yet affordable and easily applied priming substances for their efficacy. In the context of arid agriculture, developing low-cost seed-based strategies to strengthen stress tolerance acquisition in safflower could offer significant benefits. Insights into priming-mediated physiological adaptations could thus aid in designing resilient varieties for marginal growers.

Through a systematic evaluation of priming treatments, this research aims to address current knowledge gaps regarding priming-conferred stress protective mechanisms in safflower. Comprehensive analysis of growth, antioxidants, osmolytes and cellular integrity will provide insights towards developing an effective priming technique for improving the crop's drought resilience from seed to establishment.

MATERIAL AND METHODS

Seed Material:

Safflower genotype Padideh seeds collected during the previous growing season were used, with an initial seed germination rate of 96%. Safflower seeds were superficially sterilized by immersing in 1% sodium hypochlorite solution for two minutes at room temperature. They were then thoroughly washed with deionized water.

Priming Treatments:

The experiment was conducted in a completely randomized factorial design with three replications and two factors of priming method and osmotic stress level in petri dishes placed in a germinator. For priming, 75 seeds per treatment were placed in solutions of distilled water, 200 mM glutathione, 10 μ M epibrassinolide, 2% chitosan and 500 μ M folic acid. Priming was done at 25°C for 12 hours in darkness with aeration of solutions.

After priming, seeds were dried in shade at room temperature and 45% relative humidity for 24 hours until moisture content reached 8-10%. Non-primed seeds were used as control (Marthandan *et al.*, 2020).

Osmotic Stresses:

Three levels of osmotic stress including control, 4 bar with polyethylene glycol and 8 bar with polyethylene glycol were considered. 25 primed seeds from each treatment were planted in 3 petri dishes (three replications).

Germinator conditions:

Seedlings were grown in a germinator at 30°C/25°C day/night temperatures with 16 hours light and 8 hours dark and 70% relative humidity for 10 days.

Measurements:

On the 10th day after sowing, seedling samples were taken for physiological analyses. Seedling length was measured by averaging 10 seedlings in each petri dish. Then fresh leaf samples (0.5 g) were homogenized using ice extraction solution for superoxide dismutase (SOD) enzyme assay, nitroblue tetrazolium chloride solution was prepared. Absorbance of samples was read at 550 nm to determine SOD concentration (Elavarthi and Martin, 2010). For Catalase (CAT) assay, phosphate buffer and hydrogen peroxide solutions were mixed with samples and absorbance was recorded at 240 nm to calculate CAT concentration (Elavarthi and Martin, 2010).

For proline estimation, sulfosalicylic acid solution was prepared. Samples were incubated at 100°C for 30 min, ninhydrin reagent was added and absorbance was measured at 500 nm (Ábrahám *et al.*, 2010). For soluble sugars, an anthron reagent was prepared and sugars were extracted using 80% alcohol. Samples absorbance was recorded at 630 nm against standard curve (Gomez *et al.*, 2022). Bradford reagent was used to quantify soluble proteins with absorbance reading of 595 nm (Deans *et al.*, 2018). Malondialdehyde (MDA) content was estimated by incubating leaf samples in thiobarbituric acid at 95°C and reading absorbance at 532 nm (Du *et al.*, 1992).

Statistical Analysis:

Data were statistically analyzed using SAS 9.4 software including analysis of variance table and mean comparison of traits among priming treatments separately for each stress level using Duncan's test (Aghaei and Rahimi *et al.*, 2020).

RESULTS

Severe osmotic stress increased the amount of soluble leaf proteins in all priming treatments including the distilled water treatment. The highest increase in soluble leaf proteins was observed in folic acid, chitosan and epibrassinolide treatments which did not differ significantly from each other and on average showed 35% more protein compared to the non-primed treatment. In addition, the folic acid treatment

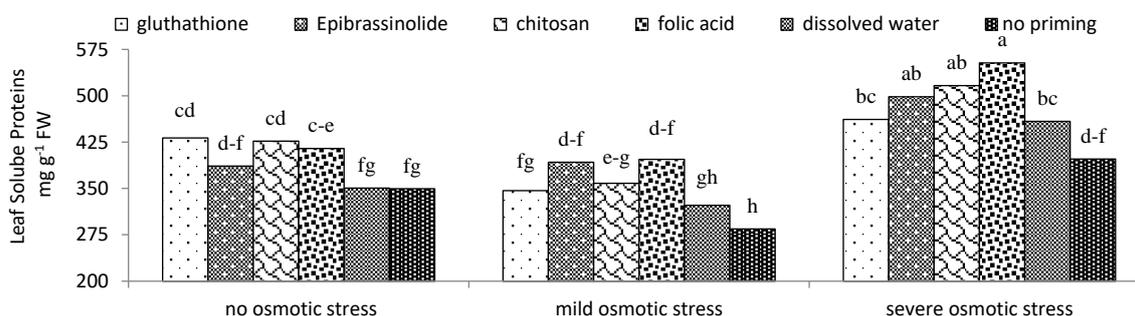


Fig. 1. Osmotic stress interaction × different priming methods on leaf soluble proteins in safflower seedling. showed higher soluble leaf proteins than the distilled water treatment and the glutathione treatment showed higher levels than the control. However, the glutathione treatment did not differ significantly from the distilled water treatment (Figure 1).

Mild osmotic stress increased leaf proline in glutathione, epibrassinolide and chitosan treatments. Severe osmotic stress increased this trait in all treatments except epibrassinolide. In non-stress conditions and both levels of osmotic stress, the chitosan treatment was superior in terms of leaf proline. However, in non-stress conditions, the chitosan treatment only had more proline compared to the control and distilled water. Also under mild stress, it did not differ from epibrassinolide but under severe osmotic stress, it was superior to all treatments in terms of proline. The chitosan treatment under mild and severe osmotic stress showed 172% and 188% more proline compared to the control, respectively (Figure 2).

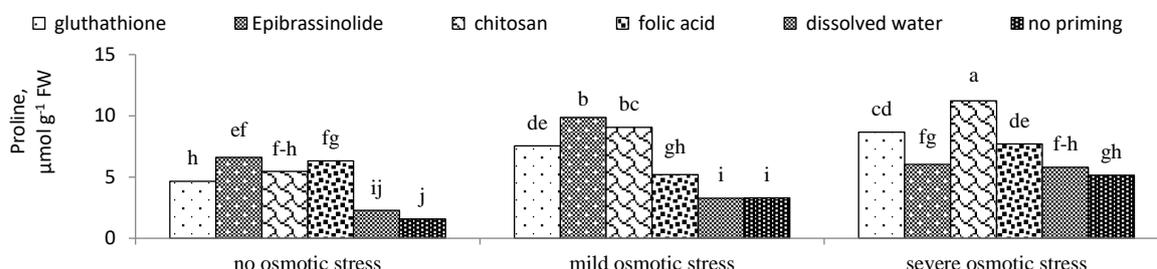


Fig. 2. Osmotic stress interaction × different priming methods on proline content in safflower seedlings.

Under non-stress conditions, all priming treatments including distilled water showed higher catalase levels compared to non-priming, although chitosan and distilled water increased it less compared to other treatments. In mild osmotic stress conditions as well, all treatments including distilled water had more catalase than the control with epibrassinolide treatment having 155% more catalase than control compared to all other treatments. Under severe osmotic stress conditions, all treatments except distilled water increased catalase, and the folic acid treatment with 207% more catalase than control was superior to all other treatments (Figure 3).

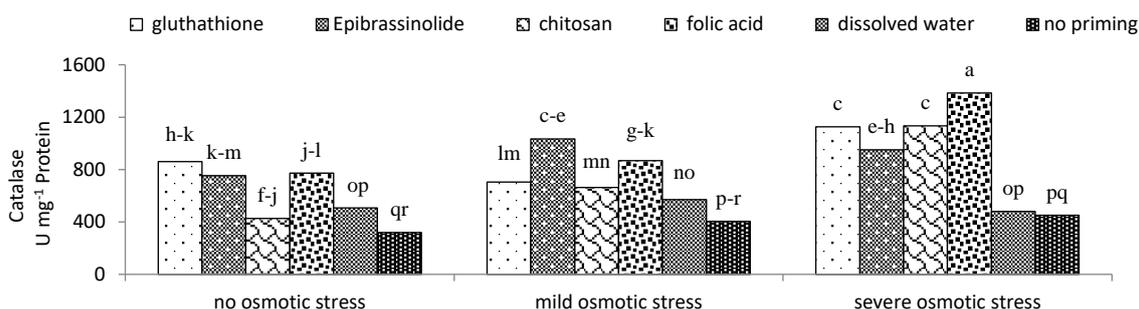


Fig. 3.- Osmotic stress interaction × different priming methods on catalase content in safflower seedlings.

Under non-stress and mild osmotic stress conditions, the distilled water treatment led to increased superoxide dismutase (SOD). Under non-stress, epibrassinolide and glutathione treatments on average showed 201% more SOD amounts compared to non-priming. Under mild osmotic stress, chitosan, epibrassinolide, folic acid and glutathione treatments did not differ significantly and on average had 14.7% more SOD compared to control. Under severe osmotic stress, little difference existed between treatments in terms of SOD (Figure 4).

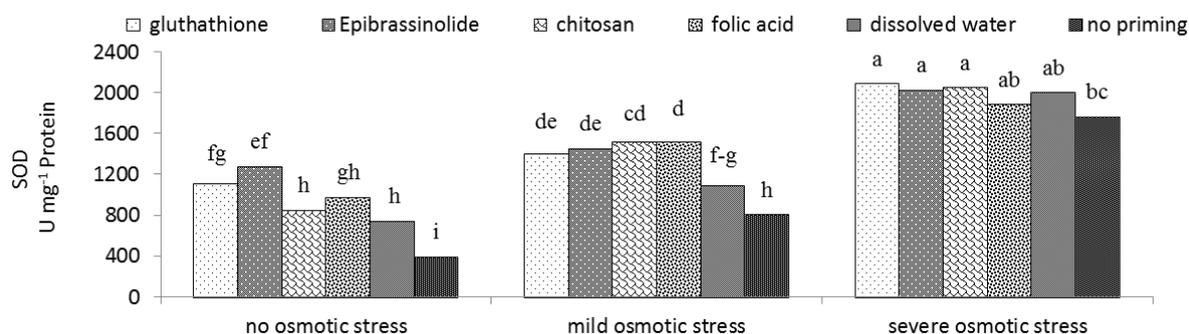


Fig. 4. Osmotic stress interaction × different priming methods on SOD content in safflower seedlings.

Under non-stress conditions, glutathione and epibrassinolide priming treatments did not show superiority over distilled water in terms of soluble leaf sugars but like other treatments had more sugars than non-priming.

The highest amounts belonged to folic acid and chitosan treatments which on average had 48.6% more soluble sugars compared to control. Under mild stress only glutathione and epibrassinolide had more soluble sugars than control which was around 14.5% for both. These two treatments also had more soluble proteins than distilled water at both stress levels (Figure 5).

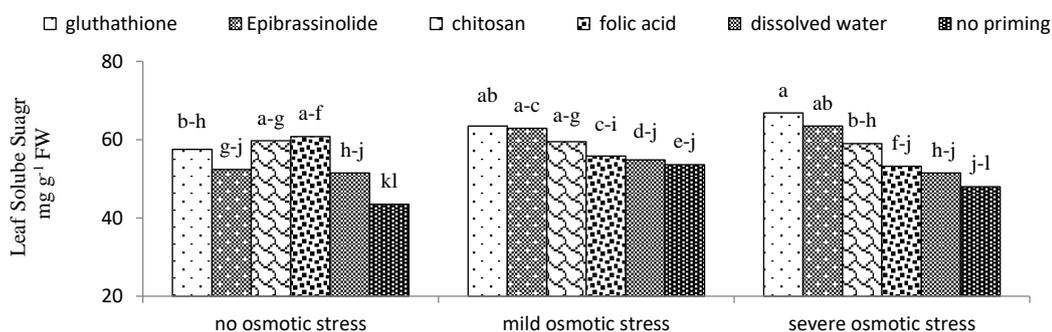


Fig. 5. Osmotic stress interaction × different priming methods on leaf soluble sugars content in safflower seedlings.

Osmotic stress led to increased malondialdehyde (MDA). Under non-stress conditions, control and distilled water treatments and under mild and severe stress, control treatment had the highest MDA amounts. Under non-stress and mild stress, epibrassinolide treatment did not differ from control in terms of MDA but other treatments had lower amounts compared to control. Under non-stress, glutathione had 19.6% lower MDA than control. Under mild stress, folic acid and chitosan on average had 21.5% lower MDA and under severe stress, control compared to glutathione and chitosan had on average 15% lower MDA amounts (Figure 6).

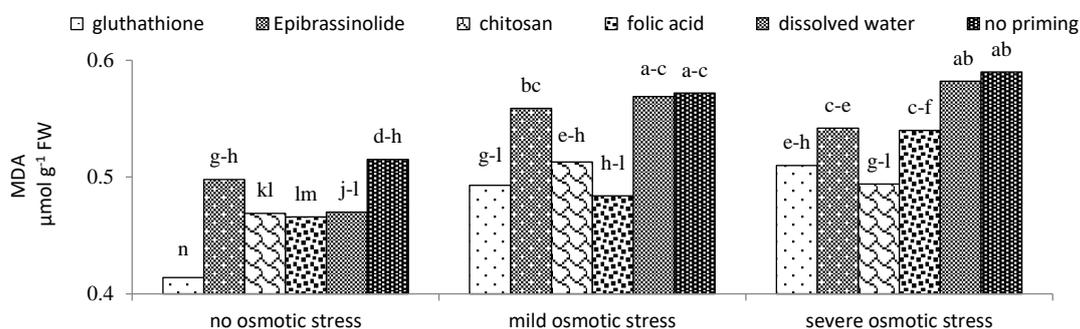


Fig. 6. Osmotic stress interaction × different priming methods on MDA content in safflower seedlings, NOS, MOS and SOS: no osmotic stress, mild osmotic stress, and severe osmotic stress, respectively.

Under non-stress conditions, control, distilled water and folic acid treatments did not differ in terms of seedling length. Chitosan had lower seedling length compared to these three treatments. Glutathione and epibrassinolide on average with 6.9% more seedling length than control had highest amounts. Under mild stress, folic acid, chitosan and epibrassinolide were superior to control. Under severe stress, unlike non-stress and mild stress conditions, distilled water had 20% more seedling length compared to control, and chitosan with 25% more length than control was slightly superior to other treatments (Figure 7).

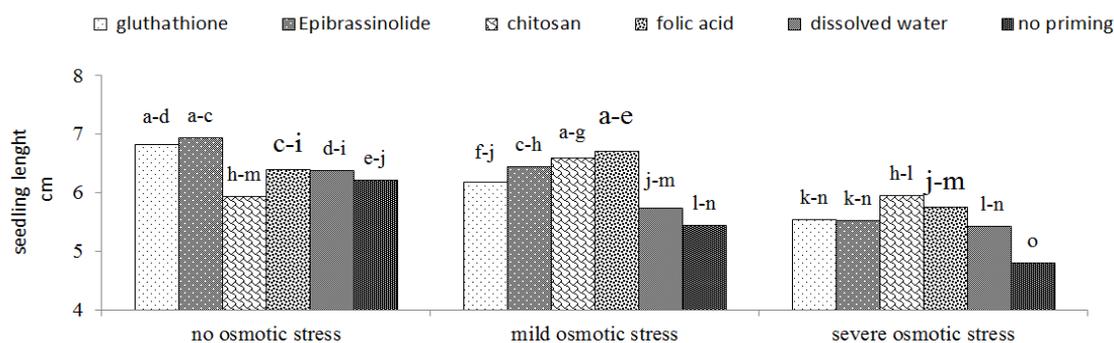


Fig. 7. Osmotic stress interaction × different priming methods on in safflower seedlings.

DISCUSSION

The results of this laboratory study show that using priming treatments of safflower seeds including glutathione, epibrassinolide, chitosan and folic acid can significantly reduce the harmful effects of osmotic stress due to water deficiency in the early growth and development stages of seedlings. These results are consistent with previous studies in the field of seed priming using the mentioned materials. For example, the findings of Hidangmayumet *et al.* (2020) show that chitosan can be helpful in priming maize seeds. Also, Budran *et al.* (2023) regarding priming cotton seeds with folic acid, Saeed *et al.* (2023) regarding priming corn seeds with glutathione and also Aghae (2020) and Karimi et al reported similar results regarding priming cotton seeds with epibrassinolide.

The glutathione treatment significantly increased the activity of the antioxidant enzyme SOD (Figure 4) and reduced oxidative stress. The epibrassinolide treatment also showed the highest amount of superoxide dismutase (Figure 4), indicating its effectiveness in strengthening the plant's defense system. The chitosan treatment was very effective in increasing proline levels (Figure 2) and the folic acid treatment strengthened the hydrogen peroxide degradation pathway by significantly increasing catalase (Figure 3). Physiological trait analysis by other researchers has shown that these priming treatments increase drought resistance by reinforcing the antioxidant defense system, regulating osmotic homeostasis and providing the necessary energy for vital processes (Marthandan *et al.*, 2020). Based on the results of this study, it can also be concluded that each of these treatments helps increase plant resistance through different mechanisms. Therefore, the idea emerges that the simultaneous use of these treatments can cause greater cooperation of defense mechanisms to increase seedling tolerance to osmotic stress ((Akbari *et al.*, 2020), which requires confirmation through new studies with combined treatments (Marthandan *et al.*, 2020).

Based on the results, chitosan and epibrassinolide played a more important role in regulating osmosis than other treatments. The chitosan treatment produced significantly higher amounts of proline at all stress levels (Figure 2), indicating its ability to regulate osmosis. Also, the epibrassinolide treatment had the highest amount of soluble sugars under non-stress and mild stress conditions (Figure 5), which plays an important role in providing energy and regulating osmotic pressure. Therefore, based on the working mechanism of these two treatments, chitosan and epibrassinolide played a more important role in regulating osmotic homeostasis among the treatments studied.

Based on results, folic acid and epibrassinolide had the greatest effect on reducing MDA levels through changes in catalase and SOD enzymes (Figures 3, 4 and 6). The folic acid treatment strengthened H₂O₂ degradation by significantly increasing catalase (Figure 3). Also, the epibrassinolide treatment with the highest SOD level better controlled oxidative stress and consequently reduced MDA (Figures 4 and 6). Similar studies also show that epibrassinolide and folic acid had the greatest impact on reducing lipid peroxidation through reinforcing the antioxidant system and cooperation of SOD and catalase enzymes (Burdan *et al.*, 2023, Aghae *et al.*, 2020).

In terms of providing the necessary energy through production of soluble sugars, the epibrassinolide and folic acid treatments performed better (Figure 5). The epibrassinolide treatment produced the highest amount of soluble sugars under non-stress and mild stress conditions (Figure 5), indicating better cell energy provision under osmotic stress. Also, the folic acid treatment under non-stress conditions after epibrassinolide had the highest level of soluble sugars (Figure 5), which plays an important role in providing energy for cellular activities. Similar studies also show that in terms of providing the necessary energy through sugars, epibrassinolide and folic acid treatments are more effective (Tanveer *et al.*, 2019).

Based on a complete analysis of the results, the chitosan treatment showed the best results in improving osmotic stress conditions for seedlings. This treatment significantly produced the highest amount of proline (Figure 2) for osmotic regulation. It also performed satisfactorily in controlling oxidative stress and providing energy through increasing SOD and CAT enzymes and soluble sugars (Figures 3, 4 and 5). Therefore, considering the simultaneous positive effects of chitosan on osmotic regulation, the antioxidant system and energy provision, it can be said that this treatment showed the most successful performance in improving osmotic stress conditions in seedlings using a set of effective mechanisms (Mustafa *et al.*, 2022). Under mild stress, the chitosan treatment along with two other treatments showed the highest seedling length (about 20% higher than control) (Figure 7). Under severe osmotic stress, chitosan with 25% more dry matter than control had a slightly higher seedling length than other treatments (Figure 7).

The results obtained from this study can significantly confirm the initial hypothesis. Distilled water and glutathione by increasing the SOD level strengthened the antioxidant defense system. Epibrassinolide and chitosan were effective in osmotic regulation through high production of proline and sugars. Folic acid was also the energy provider under non-stress conditions through higher soluble sugar production. Therefore, the results were able to largely confirm the initial hypothesis about the different mechanisms of these treatments.

Further work is needed to fully elucidate the mechanisms of action and potential for combined priming treatments. Gene expression and histological analyses may provide insights into how different priming agents modulate stress tolerance pathways at the molecular and cellular levels. Evaluating priming with alternative compounds like putrescine and ascorbic acid, which target diverse mechanisms, could have additive benefits. Addressing limitations such as genotype variability and fluctuating stress conditions would help validate these findings. Continued research optimizing priming protocols and evaluating field performance is important to realize the agronomic potential of this promising approach.

CONCLUSION

In summary, based on the results obtained from this study, it can be suggested that priming carnation seed treatments using glutathione, epibrassinolide, chitosan and folic acid can significantly reduce the negative effects of osmotic stress due to water deficiency on seedling growth and physiology in early stages. These treatments act through different mechanisms such as strengthening antioxidant immunity, regulating osmosis and providing energy. However, chitosan performed better due to its effect on several mechanisms. Therefore, using chitosan for carnation seed priming can be an effective solution to reduce the effects of osmotic stress in farms. However, more experiments are needed to conclusively validate the results.

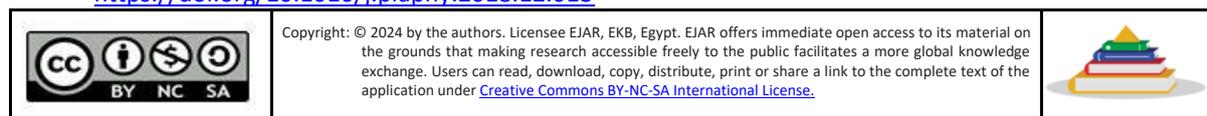
Funding: Not applicable.

Conflict of Interest: The authors declare no conflict of interest

REFERENCES

- Ábrahám, E., Hourton-Cabassa, C., Erdei, L., Szabados, L. (2010). Methods for determination of proline in plants. *Methods in molecular biology*, 63(9), 317-331. https://doi.org/10.1007/978-1-60761-702-0_20
- Aghaee, P., Rahmani, F. (2020). Seed priming with 24-epibrassinolide alters growth and phenylpropanoid pathway in flax in response to water deficit. *Journal of Agricultural Science and Technology*, 22(4), pp.1039-1052. <http://dorl.net/dor/20.1001.1.16807073.2020.22.4.2.4>
- Akbari, G. A., Heshmati, S., Soltani, E., Amini Dehaghi, M. (2020). Influence of seed priming on seed yield, oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) grown under water deficit. *International Journal of Plant Production*, 14(1), 245-258. <https://doi.org/10.1007/s42106-019-00081-5>
- Budran, E. G., Hassan, N. M., El-Bastawisy, Z. M., El-Harary, E. H., Nemat Alla, M. M. (2023) Enhancing drought tolerance of flax and improving oil quality features by priming seeds in folic acid. *Agrochimica: International Journal of Plant Chemistry, Soil Science and Plant Nutrition of the University of Pisa*, 67(1), 45-65. <https://doi.org/10.3390/43>.
- de Almeida Silva, M., Santos, H. L., de Sousa Ferreira, L., Silva, D. M. R., dos Santos, J. C. C., de Almeida Prado Bortolheiro, F. P. (2023). Physiological Changes and Yield Components of Safflower (*Carthamus tinctorius* L.) Lines as a Function of Water Deficit and Recovery in the Flowering Phase. *Agriculture*, 13(3), 558. <http://dx.doi.org/10.3390/agriculture13030558>
- Deans, C. A., Sword, G. A., Lenhart, P. A., Burkness, E., Hutchison, W. D., Behmer, S. T. (2018) Quantifying plant soluble protein and digestible carbohydrate content, using corn (*Zea mays*) as an exemplar. *Journal of Visualized Experiments*, 138(4), 58164. <https://doi.org/10.3791/58164>

- Du, Z., Bramlage, W. J. (1992) Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *Journal of Agricultural and Food Chemistry*, 40(9),1566-1570. <https://doi.org/10.1021/jf00021a018>
- Elavarthi, S., Martin, B. (2010). Spectrophotometric assays for antioxidant enzymes in plants. *Methods in Molecular Biology*, 6(39), 273-280. https://doi.org/10.1007/978-1-60761-702-0_16
- Gomashe, S. S., Ingle, K. P., Sarap, Y. A., Chand, D., Rajkumar, S. (2021). Safflower (*Carthamus tinctorius* L.): An underutilized crop with potential medicinal values. *Annals of Phytomedicine*, 10(1), 242-248. <http://dx.doi.org/10.21276/ap.2021.10.1.26>
- Hassan, N. M., El-bastawisy, Z. M., Badran, E. G., Hamady, E. M. (2016). Role of stigmasterol and folic acid in improving the growth and yield of flax under drought. *Scientific Journal for Damietta Faculty of Science*, 6(1), 40-48. <https://doi.org/10.21608/sjdfs.2016.194531>
- Hidangmayum, A., Dwivedi, P., Kumar, P., Upadhyay, S. K. (2023). Seed priming and foliar application of chitosan ameliorate drought stress responses in mungbean genotypes through modulation of morpho-physiological attributes and increased antioxidative defense mechanism. *Journal of Plant Growth Regulation*, 42(10), 6137-6154. <https://doi.org/10.1007/s00344-022-10792-1>
- Kashif, M. S., Nawaz, M., Wahla, A. J., Shahbaz, M., Ali, L., & Chaudhary, M. T. (2021). Effect of different seed priming techniques on germination and yield of wheat at different sowing dates. *Egyptian Journal of Agricultural Research*, 99(1), 118-127.
- Marthandan, V., Geetha, R., Kumutha, K., Renganathan, V. G., Karthikeyan, A., Ramalingam, J. (2020) Seed priming: a feasible strategy to enhance drought tolerance in crop plants. *International journal of molecular sciences*, 21(21), 8258. <https://doi.org/10.3390/ijms21218258>
- Mustafa, G., Shehzad, M. A., Tahir, M. H. N., Nawaz, F., Akhtar, G., Bashir, M. A., Ghaffar, A. (2022) Pretreatment with chitosan arbitrates physiological processes and antioxidant defense system to increase drought tolerance in alfalfa (*Medicago sativa* L.). *Journal of Soil Science and Plant Nutrition*, 22(2), 2169-2186. <https://doi.org/10.1007/s42729-022-00801-3>
- Saeed, F., Kausar, A., Ali, Q., Akhter, N., Tehseen, S. (2023) Impact of Combined Glutathione and Zn Application for Seed Priming in Ameliorating the Adverse Effects of Water Stress on Maize Seed Germination Attributes, Metabolite Levels, and Seedling Vigor. *Gesunde Pflanzen*, 75 (2), 2147-2168. <https://doi.org/10.1007/s10343-023-00831-6>
- Saeed, E. M., & Mohammed, H. F. (2023). Enhancement of salinity stress tolerance in cumin (*Cuminum cyminum* L.) using seed priming with Amla extract and NaCl. *Egyptian Journal of Agricultural Research*, 101(1), 200-212.
- Tanveer, M., Shahzad, B., Sharma, A., Khan, E. A. 2019. 24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry*, 135(2), 295-303. <https://doi.org/10.1016/j.plaphy.2018.12.013>



تقييم إمكانية الحد من تأثيرات الضغط الاسموزي على نمو الباقلاء من خلال معالجة البذور بالجلوتاثيون والإبيبراسينوليد والكيوتوزان وحامض الفوليك

ناصر عبد العازار , حسين زاهدي* , يونس شرقي , سيدعلى محمد مدرس سانافي , أكبر عليبور

قسم الهندسة الزراعية ومركز بحوث المحاصيل المتكاملة ، فرع إسلام شهر ، جامعة آزاد الإسلامية ، إسلام شهر ، إيران

* بريد المؤلف المراسل hzahedi2006@gmail.com

كان الهدف من الدراسة مقارنة تأثير طرق المعالجة المسبقة للبذور على فيزيولوجيا فسائل البتونيا تحت ظروف الضغط الاسموزي. تم تعريض بذور البتونيا لمعالجات مسبقة تشمل الماء المقطر وحامض الفوليك والجلوتاثيون والإبيبراسينوليد والكيوتوزان ثم تعرضها لضغوط اسموزية متضمنة عدم وجود ضغط الكي اسموزي وضغط الكي اسموزي خفيف (4 بار) وضغط الكي اسموزي شديد (8 بار) للإنبات ونمو الفسائل. أدى الضغط الاسموزي الشديد إلى زيادة البروتينات الذائبة في الورقة، ولوحظ هذا الزيادة في كافة معالجات البذور باستثناء الماء المقطر، وكانت أعلى نسبة زيادة متوسطة وقدرها 35% مرتبطة بمعالجات حامض الفوليك والكيوتوزان والإبيبراسينوليد. أدى الضغط الاسموزي الخفيف إلى زيادة البرولين في الورقة بالنسبة لمعالجات الجلوتاثيون والإبيبراسينوليد والكيوتوزان. في الضغط الشديد، لوحظت زيادة البرولين في كافة المعالجات باستثناء الإبيبراسينوليد. وكانت معالجة الكيوتوزان هي الأكثر تفوقاً من حيث البرولين تحت كافة ظروف الضغط. كما ازدادت انزيم كاتالاز أيضاً في كافة معالجات البذور باستثناء الماء المقطر تحت ظروف الضغط. وفي الضغط الشديد، أظهرت معالجة حامض الفوليك أعلى معدل زيادة في انزيم كاتالاز. كما تأثرت كمية انزيم إس أو دي بالمعالجات المختلفة تحت ظروف عدم الضغط والضغط الخفيف. كذلك تأثرت السكريات الذائبة بظروف الضغط الاسموزي المختلفة لدى جميع المعالجات. وانخفضت مؤشر MDA بمعالجتي الجلوتاثيون والكيوتوزان تحت ظروف الضغط. كما تأثر طول النبات أيضاً بالمعالجات، حيث أظهرت معالجة الكيوتوزان أعلى طول بنسبة 25% مقارنة بالكنترول تحت الضغط الشديد. يمكن استنتاج أن معالجة الكيوتوزان أظهرت أفضل أداء في تحسين تحمل النبات للضغط الاسموزي.

الكلمات المفتاحية: الضغط الاسموزي، المعالجة المسبقة للبذور، تنظيم الاسمولية، مضادات الأكسدة، الطاقة الخلوية.