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(Research Article)

Glycine betaine seed priming to induce salt stress tolerance in durum wheat (*Triticum durum* Desf.)

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Abstract

Salinity stress is an important environmental factor that limits plant growth and productivity in the world. The effect of seed priming with glycine betaine (GB) on the germination of four durum wheat varieties (Salim, Maâli, Monastir and Carioca) under salt stress conditions induced by sodium chloride (NaCl) were investigated. Three concentrations of GB (0, 50 and 100 mM) were tested under three levels of salt stress (0, 100 and 200 mM). The main results obtained showed significant effects of treatments and varieties on most studied traits. Salt stress had a negative effect on the germination and growth of wheat seedlings. However, seed priming with GB significantly improved seed germination under both no stress and salt stress conditions. Under severe salt stress conditions (200 mM NaCl), the highest values of germination percentage (65.5%), mean daily germination (0.825), vigor index (119.566), shoot length (0.568 cm) and root length (3.05 cm) were obtained using 50 mM GB. Consequently, the results of this study indicate that GB seed priming at 50 mM can to help durum wheat grow in salt-stress conditions.

Keywords: Durum wheat; Salt stress; Seed priming; Betaine glycine

1. Introduction

Cereals are staple crops and contribute significantly to global food security [1]. On 219 million square meters of cultivated land, 713 million tonnes of wheat are produced every year. In Tunisia, food security depends on cereal supply. The Tunisian diet is based on cereal products and their derivatives. They are used in different local dishes such as Couscous, Borghol and Bsissa.... Durum wheat is the main cereal crop and plays an important role in the food supply. It accounts for 51% of consumed cereals and it provides 54% of caloric intake and 64% of protein needfor the population [2].In 2020, it was grown on 547,907 ha, representing more than 46% of the total cereal area (1,168,961 ha) [3]. However, durum wheat production faces biotic and abiotic constraints that can decrease its production byabout 29% [4,5].

Soil salinity is a considerable problem in the many regions of Tunisia where durum wheat is mainly grown [6].This stress reduced grain germination, plant growth and development, causing significant yield losses [7].It inhibits the capacity of the grain to imbibe water by the low water potential in the soil solutions [8]. Consequently, it inhibits radical and plumule from penetrating the seed coat and prolonging germination time [9].

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Wheat germination and seedlings growth characteristics, such as germination percentage, germination index, shoot and root length are significant indicators to estimate salt tolerance [10,11].

Various techniques have been applied to mitigate the adverse effects of salt stress in plants such as seed priming. Priming is an effective strategy for stimulating responses to biotic and abiotic stresses [1]. It is frequently used in many African and Asian countries to ensure the simultaneous germination of different crops under unfavorable environmental conditions. It represents a pre-sowing seed treatment technique to facilitate germination [12]. Seed priming techniques are classified into hydraulic priming, osmotic priming, chemical priming, hormonal priming, nutrient priming, bio-priming and nano-priming [12]. Seed priming is effective in improving wheat tolerance to salt stress [13].

Osmotic priming is the standard priming technique. Seeds are incubated in a well aerated, low water potential solution, then washed and dried. Osmotic priming of seeds allows the seed to absorb water to enter the early stages of germination, but prevents the radicle from passing through the seed coat [14]. Various solutes such as mannitol, glycerol, glycine betaine, etc. have been used in this type of priming

Glycine betaine (GB) is an osmoprotectant that naturally accumulates in many stressed plants and plays a crucial role in plant drought tolerance [15]. It has been reported that it is an osmotic regulator of plant growth under abiotic stress, including salinity [16]. It is also used as a chemical osmoregulatory to optimise plant growth under salt stress [17]. GB acts as an osmoprotectant, enzyme activator and cell membrane stabiliser. GB has been shown to protect cells from stress by maintaining osmotic balance. Application of glycine betaine improves plant germination and growth in stressful environments [15]. Seed priming can improve germination process by enhancing metabolic activity, enhancing soluble and proline content, increasing seedling vigor under both stressed and unstressed environmental conditions [12].Seed priming with glycine betaine is a useful way to improve germination, root emergence and seedling growth, especially under stress conditions[18,19].

In view of destructive effect of salinity in wheat growth and production,the present study was carried out to assess the effect of seed priming with glycine betaine on the germination and growth of durum wheat seedlings under salt stress

2. Material and methods

2.1. Plant material

The plant material used consists of four durum wheat varieties: Salim, Maâli, Monatsir and Carioca, there characteristics are presented in the following table (Table 1).

Table 1 Morphological characteristics and origin of studied durum wheat varieties

Varieties	Origin	Morphological characteristics
Salim	Variety resulting from crossing: D92-27 made at INRAT and registered in 2007	Spike: square barbed blackish Straw: hollow Moderately resistant to lodging
Maâli	New variety registered in 2009	Spike: blackish barbed square Straw: strong and moderately resistant to lodging Very productive variety, more resistant to septoria and rust
Monastir	Variety registered in 2012	Very productive variety, widely adapted, can be grown in most areas.
Carioca	Variety registered in 2011	Early variety, more resistant to drought and very sensitive to powdery mildew.

2.2. Seed sterilization and glycine betaine seed priming

The seeds of each variety were disinfected with 12% sodium hypochlorite for 5 minutes then, rinsed 3 times with distilled water. Then, seeds were soaked in a glycine betaine solution with three concentrations: 0, 50 and 100 mM, corresponding to 0, 3.753 g/l and 7.507 g/l respectively. Priming was carried out in the dark for 12 h, followed by rinsing 3 times and then drying for 48 h on filter paper until the grains regained their initial weight.

2.3. Salt stress application and seed germination

Two saline solutions of different concentrations, 100 mM and 200 mM, were prepared by dissolving 2.92 g/l and 5.844 g/l NaCl in distilled water respectively.

Disinfected seeds were placed on two layers of filter paper in Petri dishes at a rate of 10 seeds per dish. The control dishes were soaked with distilled water and the other dishes were soaked with solutions containing different concentrations of NaCl (100 mM and 200 mM). The experiment was carried out under controlled conditions (humidity: 50%; mean temperature 22 ± 2°C day and night) in the laboratory. The experimental set-up was completely randomised, with 3 replicates for each variety and treatment.

Finally, the following nine treatments were established

T0: Control (0 mM, 0 mM GB) ;	T3: 100 mM NaCl, 0 mM GB	T6: 200 mM NaCl, 0 mM GB
T1: 0 mM NaCl, 50 mM GB ;	T4: 100 mM NaCl, 50 mM GB	T7: 200 mM NaCl, 50 mM GB
T2: 0 mM NaCl, 100 mM GB ;	T5: 100 mM NaCl, 100 mM GB	T8: 200 mM NaCl , 100 mM GB

2.4. Seed germination and seedling growth traits

- Germination percentage (GP, %)

$$GP = (\text{number of germinated grains}/\text{number of grains germinated})*100$$

- Mean daily germination (MDG)

$$MDG = \text{final emergence} / 10$$ [20]

- Vigor index (IV)

$$IV = \%\ \text{germination} * \text{mean seedling length}$$

- Coleoptile length (CL, cm)
- Shoot length (SL, cm)
- Root length (RL, cm)
- Root number (RN)

2.5. Statistical analysis

The data collected were subjected to an analysis of variance using SPSS version 20.0 statistical software. Means were compared using Duncan's test at 1% and 5%.

3. Results and discussion

3.1. Effect of seed priming with glycine betaine on durum wheat germination under salt stress conditions

The objective of this study was to evaluate the effect of osmoprming using glycine betaine on the germination and growth of durum wheat seedlings under sodium chloride (NaCl)-induced salt stress. Analysis of variance showed that salt stress and glycine betaine had highly significant effects ($p<0.001$) on all measured parameters (table 2).

Table 2 Analysis of variance (mean square and F-test) for germination percentage (GP, %), vigor index (VI), mean daily germination (MDG), shoot length (SL, cm), root length (RL, cm), coleoptile length (CL, cm) and root number (RN)

Source de variation	Ddl	GP (%)	VI	MDG	SL (cm)	RL (cm)	CL (cm)	RN
SS	2	263.215***	418.841***	265.365***	558.719***	245.890***	182.495***	75.606***
GB	2	11.973***	12 .125***	454.959***	12.159***	7.224***	29.871***	123.514***
V	3	1.822 ns	24.070***	1.993 ns	11.818***	29.910***	11.968***	14.576***
SS × GB	4	0.076 ns	1.097 ns	0.097 ns	3.210***	0.467 ns	0.825 ns	0.274 ns
SS × V	6	2.154*	13.396***	2.130 ns	9.952***	11.263***	1.963 ns	2.085 ns
GB× V	6	1.322 ns	1.271 ns	1.456 ns	0.737 ns	1.419 ns	1.233 ns	0.585 ns
SS × GB × V	12	1.137 ns	1.890*	1.211 ns	1.493 ns	1.454 ns	1.183 ns	1.698 ns

SS : saline stress; GB :glycine betaine;V :Varieties; GP : germination percentage (%); SL : shoot length (cm); RL : roots length (cm); VI : vigor index; CL : coleoptile length (cm); MDG :mean daily germination; Ddl : degree of freedom; ns : not significant; * : significant at $p<0.05$; ** : significant at $p<0.01$; *** : significant at $p<0.001$.

3.2. Germination percentage

Obtained results showed that the lowest germination percentage (GP) (58.10%) was recorded under salt stress conditions without glycine betaine addition (GB) (Figure 1). However, osmopriming with 50 Mm GB gave the high GP 96.16%, 85.91% and 65.5% under non-stress and salt stress conditions (100 and 200 mM NaCl), respectively. The results of Datta, [21] revealed that germination percentage of five wheat varieties was reduced with high NaCl concentrations. When the NaCl concentration increased above 100 mM, the germination capacity of the wheat lines was considerably reduced [22].Under water deficit conditions, Ahmed et al. [23] showed that GB enhanced GP in wheat. The highest PG (80% and 64%) were recorded with 50 and 100 mM GB concentrations respectively. GB seed priming is a useful technique to enhance seed germination and seedling growth [19]. It improves adversities of oxidative stress by ROS detoxification [24].

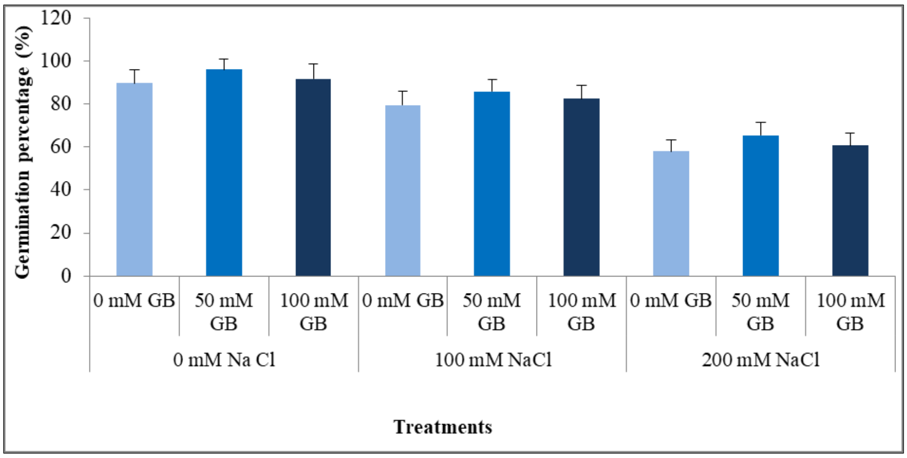

Figure 1 Effect of seed priming with glycine betaine on the germination percentage (%) of the different durum wheat varieties under salt stress conditions

3.3. Mean daily germination

Salt stress had a negative effect on mean daily germination (MDG) (Figure 2). The decrease in MDG for all varieties with increasing saline concentration can be explained by the time needed for the seed to set up mechanisms enabling it to adjust its osmotic pressure [25]. The highest values (1.14; 1.02; 0.82) were recorded with the application of 50 mM GB under different levels of salt stress (0, 100 and 200 mM NaCl).In fact, seed priming promotes uniform and synchronized plant seedling emergence under abiotic stress conditions. Physiologically, it strengthened the antioxidant (catalase, ascorbate peroxidase, superoxide dismutase) activities and promotes compatible solutes accumulation such as: proline, free amino acids, sugar…[26].

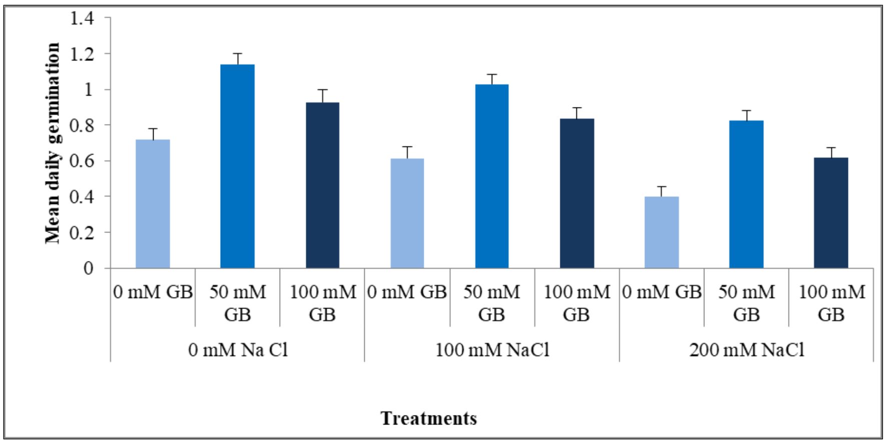

Figure 2 Effect of seed priming with glycine betaine on mean daily germination of different durum wheat varieties studied under salt stress conditions

3.4. Vigor index

As shown in figure 3, salt stress reduced vigor index (VI) of durum wheat seedlings. Under non-stress and salt stress conditions osmopriming by GB improved VI mainly using 50 mM. Ahmed et al.[23] showed that the application of 100 mM followed by 50, 25 and 10 mM GB increased VI in water-deficient wheat.

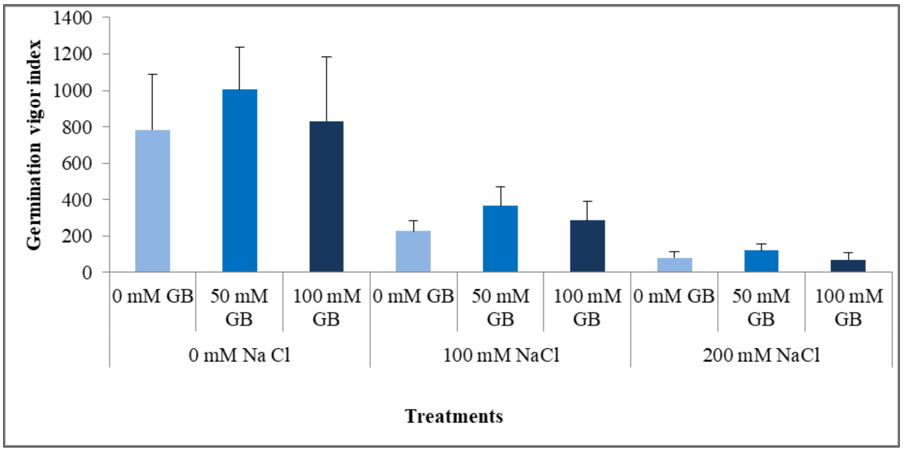

Figure 3 Effect of seed priming with glycine betaine on germination vigor index of different durum wheat varieties under salt stress conditions

3.5. Coleoptile length

Salt stress has a negative effect on coleoptile length (CL) ofstudied durum wheat varieties (Figure 4). As NaCl concentration increases, coleoptile length decreases in 16 barley varieties [27]. Under salinity, the reduction in coleoptile growth might be combined osmotic/oxidative/toxic stress that disturbance cell division and modify cell organelles structure [28].

However, highest values (2.64; 2.86 and 0.89 cm) were recorded under the three salt stress levels 0, 100 and 200 mM NaCl respectively when applying osmopriming by 50 mM GB.

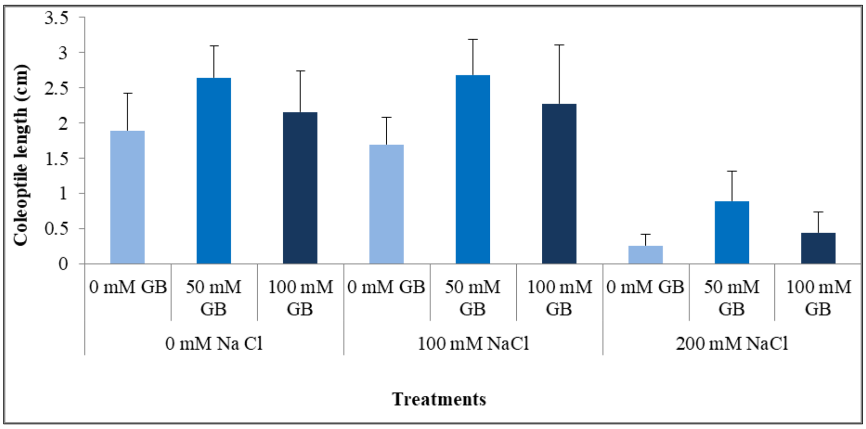

Figure 4 Effect of seed priming with glycine betaine on coleoptile length of different durum wheat varieties studied under salt stress conditions

3.6. Shoot length

The lowest shoot length (SL) (0.26 cm) was recorded under the highest salt stress level (200 mM NaCl) (Figure 5).Salt stress significantly reduced SL in five durum wheat varieties [29]. Compared with treatments without GB, the application of 50 mM GB increased this trait by 68.58% and 51.35% under salt stress conditions 100 and 200 mM NaCl respectively. This improvement is due to the positive effect of GB on photosynthesis process under salinity stress. Thus, the increase of assimilates production and their translocation into different plant parts promotes growth and biomass accumulation [30, 31].

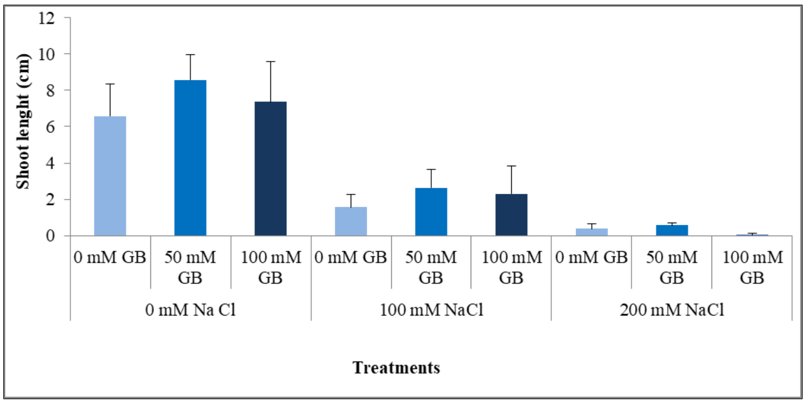

Figure 5 Effect of seed priming with glycine betaine on shoot length of different durum wheat varieties studied under salt stress conditions

3.7. Root length

Salt stress significantly (p<0,001) reduced root length (RL) (Figure 6). Rahmoune et al.[32] showed that salt stress reduced root length in *durum* and *aestivum* wheat varieties when salinity exceeded 4 g/l. Salt stress influence water absorption by roots which causes osmotic stress because of ion toxicity. The salinity stress reduces water and absorption and decreases assimilate production which reduces shoot and root growth and biomass accumulation [31, 33]. Concentrations above 50 mM NaCl significantly reduced root and shoot length in wheat[21]. Under both salt stress levels (100 and 200 mM NaCl) the highest RL (5,72 and 3,05 cm) were obtained by applying 50 mM GB. Similar results were obtained by Ahmed et al.[1].

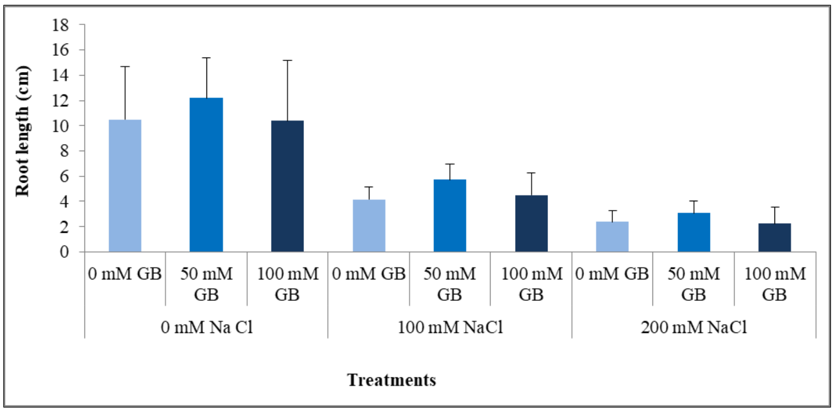

Figure 6 Effect of seed priming with glycine betaine on root length of different durum wheat varieties studied under salt stress conditions

3.8. Root number

Figure 7 shows that salt stress adversely affected root number in the different durum wheat varieties tested. Similar results were obtained by Khan et al.[34]. Salt stress inhibits lateral root development [35]. Osmopriming with GB improved the number of roots under saline stress and non-stress conditions, especially when 50 mM GB was added.

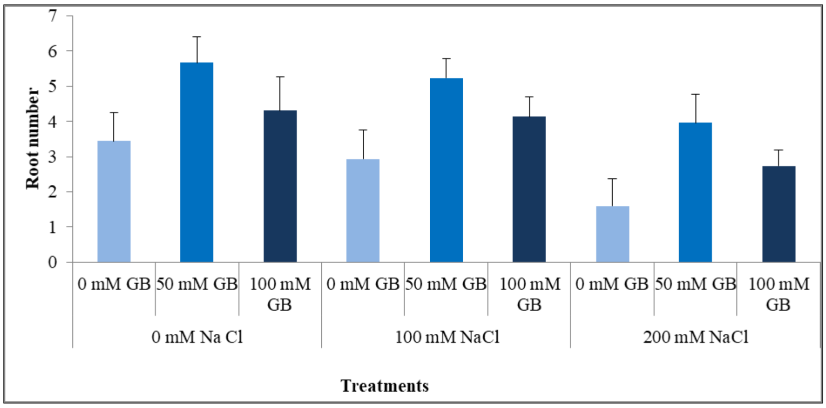

Figure 7 Effect of seed priming with glycine betaine on root number of different durum wheat varieties studied under salt stress conditions

4. Conclusion

Seed germination and seedling growth are most important stages of a plant life cycle and are mainly susceptible to salinity stress. In the present study, this stress had a negative effect on germination and growth parameters of durum wheat seedlings. Seed priming with glycine betaine significantly improved all measured traits. Also, osmopriming with 50 mM GB was more effective than with 100 mM GB.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Ahmed, N., Zhu, M., Li, Q., Wang, X., Wan, J., & Zhang, Y. (2021). Glycine betaine-mediated root priming improves water stress tolerance in wheat (Triticum aestivum L.). Agriculture, 11(11), 1127.

[2] El Felah, M.; Gharbi, M.S.; Ben Ghanem, H.; Elloumi, M. (2015). Cereals in Tunisia Between Myth and Reality; Annals of INRATT; National Institute of Agricultural Research of Tunisia: Tunis, Tunisia,;pp. 1–17.

[3] ONAGRI, National Observatory of Agriculture. (2020). Statistical Yearbook 2020

[4] Gupta, N., & Thind, S. K. (2017). Grain yield response of drought stressed wheat to foliar application of glycine betaine. Indian Journal of Agricultural Research, 51(3), 287-291.

[5] Baraket, M., Ounissi, W., Hassine, M., Chaouachi, L., Jallouli, S., Sarsu, F., ... & Trifa, Y. (2022). Pre-Field Screening of Potential Barley and Durum Wheat Mutants for Drought Tolerance. Asian Journal of Research in Biosciences, 1-11.

[6] Kim, S. H., Kim, D. Y., Yacoubi, I., & Seo, Y. W. (2018). Physiological and genetic responses of salt-stressed Tunisian durum (Triticum turgidum ssp. durum) cultivars. The Korean Journal of Crop Science, 63(4), 314-321.

[7] Seleiman, M. F., Aslam, M. T., Alhammad, B. A., Hassan, M. U., Maqbool, R., Chattha, M. U., ... & Battaglia, M. L. (2022). Salinity stress in wheat: Effects, mechanisms and management strategies. Phyton, 91(4), 667.

[8] Hameed, A.; Rasheed, A.; Gul, B.; Khan, M. (2014). Salinity Inhibits Seed Germination of Perennial Halophytes Limoniumstocksii and Suaedafruticosa by ReducingWater Uptake and Ascorbate Dependent Antioxidant System. Environ. Exp. Bot., 107, 32–38.

[9] Wang, J., Lv, P., Yan, D., Zhang, Z., Xu, X., Wang, T., ... & Li, R. (2022). Exogenous melatonin improves seed germination of wheat (Triticum aestivum L.) under salt stress. International Journal of Molecular Sciences, 23(15), 8436.

[10] Li, L., Peng, Z., Mao, X., Wang, J., Li, C., Chang, X., & Jing, R. (2021). Genetic insights into natural variation underlying salt tolerance in wheat. Journal of Experimental Botany, 72(4), 1135-1150.

[11] ZaariJabri, N., Ait-El-Mokhtar, M., Mekkaoui, F., Amghar, I., Achemrk, O., Diria, G., &Hmyene, A. (2023). Impacts of cadmium toxicity on seed germination and seedling growth of Triticum durum cultivars. Cereal Research Communications, 1-11.

[12] Saha, D., Choyal, P., Mishra, U. N., Dey, P., Bose, B., Prathibha, M. D., ... & Singhal, R. K. (2022). Drought stress responses and inducing tolerance by seed priming approach in plants. Plant Stress, 100066.

[13] Tabassum, T., Farooq, M., Ahmad, R., Zohaib, A., & Wahid, A. (2017). Seed priming and transgenerational drought memory improves tolerance against salt stress in bread wheat. Plant Physiology and Biochemistry, 118, 362-369.

[14] Heydecker, W.J., Heydecker, J., Higgins and Gulliver, K. (1973). Accelerated germination by osmotic seed treatment, Nature 246: 42-46.

[15] Raza, M. A. S., Saleem, M. F., Shah, G. M., Khan, I. H., & Raza, A. (2014). Exogenous application of glycine betaine and potassium for improving water relations and grain yield of wheat under drought. Journal of soil science and plant nutrition, 14(2), 348-364.

[16] Hanif, S., & Zia, M. (2023). Glycine betaine capped ZnO NPs eliminate oxidative stress to coriander plants grown under NaCl presence. Plant Physiology and Biochemistry, 197, 107651.

[17] Khedr, R. A., Sorour, S. G. R., Aboukhadrah, S. H., El Shafey, N. M., AbdElsalam, H. E., El-Sharnouby, M. E., & El-Tahan, A. M. (2022). Alleviation of salinity stress effects on agro-physiological traits of wheat by auxin, glycine betaine, and soil additives. Saudi Journal of Biological Sciences, 29(1), 534-540.

[18] Zhang, Q., Rue, K., & Mueller, J. (2014). The effect of glycine betaine priming on seed germination of six turfgrass species under drought, salinity, or temperature stress. Hort Science, 49(11), 1454-1460.

[19] Rakshit, A., Singh, H. B. (2018): Advances in Seed Priming. – 1st edition, Singapore: Springer Singapore.

[20] Jajarmi,V. (2008). Effect of water stress on germination indices in seven safflower cultivars (Carthamustinctorius L.). In: Proceedings of the 7th International SafflowerConference, WAGGA WAGGA, New South Wales, Australia, 1–3p.

[21] Datta, J. K., Nag, S., Banerjee, A., &Mondai, N. K. (2009). Impact of salt stress on five varieties of wheat (Triticum aestivum L.) cultivars under laboratory condition. Journal ofApplied Sciences and Environmental Management, 13(3).

[22] He, C., Yang, A., Zhang, W., Gao, Q., & Zhang, J. (2010). Improved salt tolerance of transgenic wheat by introducing betA gene for glycine betaine synthesis. Plant Cell, Tissue and Organ Culture (PCTOC), 101, 65-78.

[23] Ahmed, N., Zhang, Y., Yu, H., Gabar, A., Zhou, Y., Li, Z., & Zhang, M. (2019). Seed priming with Glycine betaine improve seed germination characteristics and antioxidant capacity of wheat (Triticum aestivum L.) seedlings under water-stress conditions. Applied Ecology and Environmental Research, 17(4), 8333-8350.

[24] Gupta, N., Thind, S. K. (2015): Improving photosynthetic performance of bread wheat under field drought stress by foliar applied glycine betaine. – Journal of Agricultural Science and Technology 17(1): 75-86.

[25] Laiche, K., Hadjou, S., &Chekara, B. M. (2022). Effect of stress on the germination of some accessions of Aegilops ventricosa L.

[26] Zhang, F., Yu, J., Johnston, C. R., Wang, Y., Zhu, K., Lu, F., Zhang, Z., Zou, J. (2015): Seed priming with polyethylene glycol induces physiological changes in sorghum (Sorghum bicolor L. moench) seedlings under suboptimal soil moisture environments. –PLoS ONE 10(10): 1-15

[27] Djerah, A., &Oudjehih, B. (2016). Effect of salt stress on the germination of sixteen varieties of barley (*Hordeum vulgare* L.). Courrier du Savoir, 20, 47-56.

[28] Baranova, E.N.; Gulevich, A.A. (2021) Asymmetry of plant cell divisions under salt stress. Symmetry, 13, 1811.

[29] Ayadi, I., & Bounegab, S. (2020). Study of intraspecific variability of tolerance to salt stress in five varieties of durum wheat (Triticum durum Desf.) at the juvenile stage (Doctoral dissertation, UNIVERSITY KASDI MERBAH–OUARGLA-).

[30] Caparrós P, Llanderal A, Hegarat E, Jiménez-Lao M, Lao MT (2020). Effects of exogenous application of osmotic adjustment substances on growth, pigment concentration, and physiological parameters of Dracaena sanderiana under different levels of salinity. Agronomy 10:125. https://doi.org/10.3390/agronomy10010125

[31] Dustgeer, Z., Seleiman, M. F., Imran, K. H. A. N., Chattha, M. U., Alhammad, B. A., Jalal, R. S., & Hassan, M. U. (2021). Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. Not Bot HortiAgrobot Cluj-Napoca 49: 12248–12248.

[32] Rahmoune, C., Sdiri, H., Meddahi, M. L., & Selmi, M. (2001). Effect of salt stress on the germination, growth and grain production of some Maghreb varieties of wheat. Science and global changes/Drought, 12(3), 167-74.

[33] Taha RS, Seleiman MF, Alotaibi M, Alhammad BA, Rady MM, Mahdi AHA (2020). Exogenous potassium treatments elevate salt tolerance and performances of Glycine max L. by boosting antioxidant defense system under actual saline field conditions. Agronomy 10(11):1741. https://doi.org/10.3390/agronomy10111741

[34] Khan, M. S., Shah, S. J., & Ullah, M. (2006). Assessment of salinity stress and the protective effects of glycine betaine on local wheat varieties. ARPN J. Agric. Biol. Sci, 9, 360-365.

[35] Cirka, M., Kaya, A. R. et Eryigit, T. (2021). Influence of temperature and salinity stress on seed germination and growth of soybean (Glycine max L.) seedlings.Legume Research-An International Journal, 44(9), 1053-1059.