Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(3) 335-340

Effect of Seed Priming on the Growth and Development of Callus from Mature Embryo of Wheat (*Triticum turgidum* subsp. *durum* Desf.)

Halima Othman*[a], Samih Tamimi[a] and Monther Sadder[b]
[a]Department of Biology, School of Science, University of Jordan, Amman, Jordan
[b]Department of Horticulture and Crop Science, School of Agriculture, University of Jordan, Amman, Jordan

(received December 27, 2023; revised May 6, 2024; accepted May 16, 2024)

Abstract. Current research is focused on finding out effective strategies to overcome the adverse effects of environmental stress conditions on plants such as drought and salinity. Seed priming is a pre-sowing seed treatment strategy frequently used to boost up stress tolerance potential of plants. Although the beneficial effects of seed priming on stress tolerance are well explored, priming influence on the in *vitro* culture of plants has not been well explored which is the main objective of this work. To achieve this objective, the influence of priming on induction and growth of wheat callus derived from mature embryos of seeds subjected to hydro-priming, kinetin priming and $CaCl_2$ priming was investigated. The results showed that all priming treatments reduced callogenesis potential and callus growth compared to those from unprimed seeds. Compared to its unprimed counterpart, the highest reduction in callus induction (66%) was recorded in the hydroprimed seeds followed by kinetin primed and $CaCl_2$ primed seeds which reduced callus induction by 33-35%. Priming also reduced callus growth. Growth of callus raised from Hydro, kinetin and $CaCl_2$ primed embryos were reduced by 18.60 %, 10.05% and 17.79% compared to control, respectively. Contrary to its effect on growth, the physiological status of callus cells was not affected by the priming treatments. Neither viability, nor MDA content of callus cells were influenced by the priming treatments. Furthermore, the priming treatments increased the membrane stability index (MSI) of callus cells. Cells from hydroprimed callus achieved a significant increase of MSI (51.6%) compared to control, which showed the lowest value (29.2 %). Cells of Kinetin and $CaCl_2$ primed groups showed lower increase for this trait (47.3, 47.7%, respectively). It is concluded that priming although reduced callus growth, it had no damaging effect on its cellular component and can be effectively used for elucidating the mechanism of priming induced stress tolerance at the cellular level.

Keywords: priming, wheat, callus, seed

Introduction

Environmental stresses have long threatened sustainable development of agricultural production. Therefore strategies for the alleviation of the negative impact of stresses on plants have been the focus of several studies. Among the different strategies tested, seed priming is considered as the most promising technique that could mitigate the harmful impact of various stresses on plants (Amir *et al*., 2024). Seed priming is the treatment of seeds with different factors of natural and synthetic origin to induce a mild dose of stress (Paparella *et al*., 2015). Seed treatments prior to germination induce endogenous plant defense mechanisms leading to subsequent in the primed plant.

The vast existing literature on the effect of priming on plants has mainly investigated how different species react to various priming treatments (Devika *et al*., 2021). However, now a new aspect of the topic is gaining interest, which can be summarized as the set of mechanisms that lead primed plants to mitigate the stress. In fact understanding the biochemical and molecular action of priming is essential for the understanding of stress resistance mechanisms in plants.

Although several studies highlighted key mechanisms underlying priming-induced tolerance in plants, most of these studies used whole plant models and mechanisms operating at the cellular level is not yet addressed and fully clarified by Amir *et al*., 2024; Giri *et al*., 2024; Lutts *et al*., 2016). Since discripencies in the priming-induced stress tolerance action at the whole plant level and those at the cellular level may exist, additional studies leading to a better understanding of the mechanisms of priming action at the cellular level is a crucial prerequiste for the understanding of priming

*Author for correspondence; E-mail: halee.othman@gmail.com

induced plant tolerance to abiotic stressors. In line with this rationale, some recent studies showed that the response to some stresses such as salinity differs between whole plants and in *vitro* cultured cells (Almansouri *et al*., 2000; Naik and Widholm, 1993).

The use of callus culture techniques has served as a very useful tool for explaining the mechanisms of stress tolerance in plants at the cellular level (Singh *et al*., 2023; Sharma and Ramawat, 2013). Callus culture has the advantage of culturing plant cells under controlled and uniform environmental conditions (Queiros *et al*., 2007; Bajji *et al*., 1998), thus avoiding complications arising from the physiological and structural variability of whole plants (Elkahoui *et al*., 2005; Bajji *et al*., 1998). The potential of this biotechnological method in eliminating obstacles that could result from variability in genetic and morphological levels associated with plants' tissues at the whole level may also prove valuable for providing information about the cellular events induced by priming treatments.

Obviously, callus cultures in *vitro* are promising model systems in this area of exploration. The calli obtained from various explants of cereals are of particular interest due to the economic value of this group of plants. Among the various explants used for callus induction in cereals, mature seed embryos are now being successfully employed for effecient callus induction (Tamimi and Othman, 2021). Nevertheless the influence of seed priming on the callus culture of wheat was not previously investigated. Accordingly, the objective of this study was to examine the effecst of primed mature embryo explants on wheat callus induction and growth using three commonly employed wheat seed priming treatments (hydropriming, $CaCl_2$ priming and kinetune priming). The possible effects of these treatments on the physiological status of the callus cells were also investigated in order to uncover possible damaging effect of the priming treatment on the cellular component of the callus culture.

Materials and methods

Plant material and seed priming. Seeds of locally used durum wheat (*Triticum turgidum* subsp. *durum* Desf.) were obtained from the School of Agriculture, University of Jordan in September 2022. Uniform wheat seeds were selected and disinfected for 10 min with 5% sodium hypochlorite (*v/v*), then, they were abundantly rinsed with distilled water. The surface sterilized seeds were primed by soaking either in sterile solution of 50 mM $CaCl_2$ or sterile 50 mg/L Kinetin solution for 10 h at room temperature. For hydropriming seeds were soaked in sterile distilled water for the same period and under the same conditions. Primed seeds were then air dried to their original moisture content and unprimed seeds were used as control.

Callus Inductiom. Primed and unprimed wheat seeds were disinfected with 40 % sodium hypochlorite solution containing few drops of Tween 80 for 15Êmin. After three washes with sterile distilled the seeds were soaked in sterile distilled water for 1- 2 h to facilitate embryo separation. The embryos were then excised from individual seeds aseptically and transferred to sterile callus induction medium (15 mL) in 90 mm Petri plates. Each petri dish had 10 embryos with 4 replicates per treatment. The callus induction medium contained 4.3 g/L MS basal salts supplemenyed with 20 g/L sucrose, 0.5 g/L glutamine,100 mg/L ascorbic acid, 0.1 g/L casein hydrolysate, 1 mL/L MS vitamin, 3 mg/L 3,6-dichloro-2- methoxy benzoic acid (dicamba), and 1 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D). Gelrite (Gelrite, Sigma Aldrich). 2 g/L was added as a solidifying agent and the pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 15 min.

All cultures were incubated at 25 °C in the dark and checked daily for callus growth and development. Data reorded were callus induction time (days taken for callus initiation), callus fresh weight determined 4 weeks after incubation and callus induction frequency estimated four weeks after culture according to Islam *et al*. (2005) using the the equation.

Callus induction frequency (%) =100* (number of embryos producing callus)/(total number of embryos cultured) per Petri plate.

Callus viability. For the determination of callus cell viability, the TTC method (Lutts *et al*., 2004) was applied. 100 mg of 4 weeks old callus were rinsed in deionized water containing 0.05% Tween-20 and then incubated at 30 °C in the dark in tubes containing 5.0 mL of 0.5% 2,3,5- triphenyltetrazolium chloride in 50 mM K2HPO4 (pH 7.0) for 15 h. The samples were then filtered and washed with distilled water, incubated in 3 ml of 94% ethanol at 80°C for 5 min. Samples were vortexed then centrifuged at 5,000 × g for 1 min and absorbance of the supernatant of each sample was measured at 500 nm. viability was rated according to Mamdouh and Smetanska (2022); absorbance 500 =

0.05 cells are non-viable; absorbance 500 between 0.05 and 0.15: cells have low viability and Absorbance 500 higher than 0.15 cells are viable.

Lipid peroxidation. Lipid peroxidation was determined by estimating the total amount of malondialdehyde (MDA) contents, according to Heath and Packer (1968) Fresh callus (1 g) was homogenized in 10 mL of 0.25% thiobarbituric acid (TBA) in 10% trichloracetic acid (TCA). The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000×*g* for 10 min the absorbance of the supernatant was read at 532 nm and at 600 nm for correction of nonspecific turbidity. The MDA content was calculated according to its extinction coefficient of 155/mM/cm and expressed as μmol/g FW.

Membrane Stability Index. The Membrane stability index (MSI) was determined in terms of relative electrolytic leakage according to the method of Sairam (2002). Callus tissue of equal weight were incubated for 24 h in a test tube containing 10 mL of distilled water at room temperature (25 °C) and the initial electrical conductivity (EC1) was measured after the incubation period. Then, the samples were placed at 100 °C over water bath for thirty minutes after which the final electrical conductivity (EC2) was measured when cooled to room temperature. The MSI was calculated as proposed by Sairam (2002) MSI (%) = [1 - (C1 /C2)] × 100.

Results and Discussion

In this study embryos of primed and unprimed seeds were excised and cultured on callus induction media. Callus were initiated from all priming treatments within 2 to 7 days but the shortest time required for callus induction was observed in unprimed wheat embryos which occurred after 2 days, 2 to 3 days from kinetin primed embryos, 2 to 4 days from $CaCl_2$ primed embryos and 5 to 6 days from hydro-primed embryos following culture. At the end of the culture period, the percentage of callus formation was around 90% in the unprimed embryo, followed by $CaCl_2$ primed (57.32 %), kinetin primed (55.83%) and it reached 23.75% in hydro-primed embryos (Table 1).

The callus growth was also compared to their fresh weight. Mean callus fresh weight showed that the highest growth of callus was observed from unprimed embryos (101 mg) compared to callus of hydro-primed, kinetin primed or $CaCl_2$ primed (82.23 mg, 90.85 mg and 83.03 mg respectively). These values showed that priming treatments mitigate callus induction compared to unprimed embryos cultures.

Table 1. The effect of seed priming treatments on callus induction parameters, (values are means of four replicates ± se)

Priming treatment	Callus induction time (Days)	Frequency of callus induction (%)
Unprimed	2	86.93 ± 4.879
Hydro-primed	5-6	23.75 ± 3.75
Kinetin primed	2-3	55.83 ± 4.167
$CaCl_2$ primed	2-4	57.32 ± 2.321

The callus cell viability test was conducted by using TTC (2.3.5-triphenyl tetrazolium chloride) for 3 replicates of 30 days callus. The results of viability are presented in Table 2 where unprimed, kinetin and $CaCl_2$ callus were viable while the viability degree was the lowest on callus originated from hydro- primed seeds.

In this study, we investigated the effect of priming on membrane stability was investigated (Fig. 2). Of callus cells. The value of the membrane stability index (MSI) was calculated according to the electrolyte leakage test. MSI was significantly better in hydro-primed seeds (51.6%) compared to unprimed control (29.2%), while it was increased insignificantly in kinetin and $CaCl_2$, 47.3 and 47.7% respectively.

MDA contents are usually used to evaluate membrane damage. In all priming treatments MDA content was the highest in callus originating from un-primed seeds (0.2 μM) while in the $CaCl_2$ primed callus, MDA content was less than in other priming treatments (0.1 μM).

Plant tissue culture has been applied to develop novel systems for studying the responses of plants to different

Table 2. Cell viability test of callus culture with different priming treatment after 30 days of culturing

Priming treatment	Absorbance at 500 nm/100mg fresh callus tissue (Mean ±SE)	Viability degree
Unprimed	0.9108 ± 0.1714	Viable
Hydro	0.03179±0.0002105	Low viability
Kin	0.7842 ± 0.04319	Viable
$CaCl_2$	0.9582 ± 0.1335	Viable

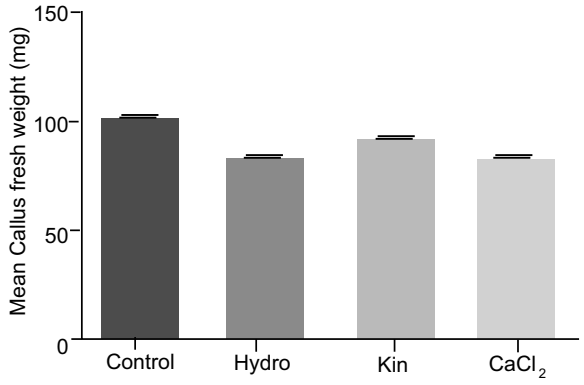

Fig. 1. Mean callus fresh weight per mg after 30 days of callus culture.

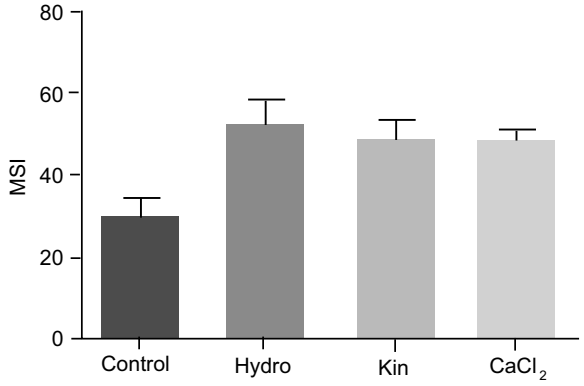

Fig. 2. The effect of different priming treatments on MSI ($P<0.05$).

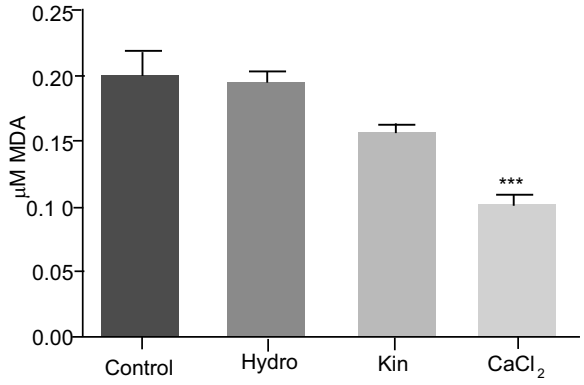

Fig. 3. MDA content in different priming treartments (µM/100mg fresh weight callus), $P<0.05$.

agents at the cellular level. The success of these studies largely depends on the suitability of the in *vitro* culture used. Several studies have shown that various factors such as explant type, explant pretreatment and medium composition are significant and influential factors for the in *vitro* growth of plant cells (Pasternak and Steinmacher, 2024). Explant pretreatment has been shown to have a profound effect on the degree of callus formation frequency and growth performance even when cultures were grown under uniform conditions. The relative importance and influence of seed priming treatment on callus growth and development from mature seed embryos are largely unknown.

Auxin and cytokinin levels, both endogenous and exogenous,is well documented on having a major role in callus formation in a variety of plant species. In general, an intermediary concentrations of auxin and cytokinin enhances callus induction, while a high auxin-to-cytokinin or cytokinin-to-auxin concentration stimulates root and shoot development (Skoog and Miller, 1957). Our results could also be related to the presence of high endogenous cytokinin (kinetin primed) relative to unprimed and other priming treatments.

Results of our present study also showed a decline in callus induction in $CaCl_2$ primed seeds. This reduction is likely due to the accumulation of certain ions. Amine *et al*. (2013) revealed that the apply $CaCl_2$ in the external media of callus culture promotes K+ uptake and beyond this, calcium chloride adversely affected K+ accumulation in tissue and ultimately callus growth was reduced. Ýn addition, high calcium in callus media found to have a negative impact on growth of calli. Such typical calcifugic behavior might be related to insufficient compartmentation orphysiological inactivation of calcium (precipitation of calcium oxalate) (Marschner, 1995).

However, calcium plays a major role in plant development and response to stresses (Niu *et al*., 2017) and seed germiation (Kong *et al*., 2015). Presence of calcium in primed seeds enhanced onset of metabolic activities (Zhang *et al*., 2020; Wang *et al*., 2016) and regule the activities of antioxidents and certain hormones during the germination process (Anaya *et al*., 2018; Rahman *et al*., 2016; Hamayun *et al*., 2015; Zhang *et al*., 2014). In addition, Ca^{2+} signaling plays a vital role in cell wall integrity and alleviating the inhibition of salt stress on cell growth (Feng *et al*., 2018). The research results

regarding MDA and MSI are in agreement to theses findings.

Conclusion

In conclusion, the results of this study suggested that Kinetin and $CaCl_2$ priming pretreatment of seeds had little negative influence on callus culture derived from the mature embryos of the treated seeds compared to unprimed or hydro-primed control seeds. These findings suggest that the callus culture protocol used in this study can be equally effective for callus culture from both primed and unprimed seeds. This is supported by the finding that Kinetin and $CaCl_2$ priming had no toxic effects on the cells of the developing callus besides having no significant negative influence on membrane stability emphasizing the lack of physiological impact on the developing callus.

Conflict of Interest. The authors declare they have no conflict of interest.

References

Almansouri, M., Kinet, J.M., Lutts, S. 2000. Physiological analysis of salinity resistance in *Triticum turgidum* var. *durum* Desf.: Callus versus whole plant responses. *In: Durum Wheat Improvement in the Mediterranean Region: New Challenges*, C. Royo, M. Nachit, N. Di Fonzo, J.L. Araus (eds.), pp. 263 265, CIHEAM, Zaragoza, Spain.

Amin, N.U., Sanga, G., Ara, N., Shah, S.H., Ullah, F. 2013. Effect of various concentrations of calcium chloride on callus growth and potassium nutrition of calli cultures of potato (*Solanum tuberosum*). *Pakistan Journal of Botany*, **45:** 209-214.

Amir, M., Prasad, D., Khan, F.A., Khan, A., Ahmad, B., Astha. 2024. Seed priming: An overview of techniques, mechanisms and applications. *Plant Science Today*, **11:** 553-563.

Anaya, F., Fghire, R., Wahbi, S., Loutfi, K. 2018. Influence of salicylic acid on seed germination of *Vicia faba* L. under salt stress. *Journal of the Saudi Society of Agricultural Science*, **17:** 1-8.

Bajji, M., Kinet, J.M., Lutts, S. 1998. Salt stress on roots and leaves of *Atriplex halmus* L. and their corresponding callus culture. *Plant Science*, **137:** 131-142.

Devika, O.S., Singh, S., Sarkar, D., Barnwal, P., Suman, J., Rakshit, A. 2021. Seed priming: A potential supplement in integrated resource management under fragile intensive ecosystems. *Frontiers in Sustainable Food Systems*, **5:**654001.

Elkahoui, S., Hernandez, J.A., Abdelly, C., Ghrir, R., Limam, F. 2005. Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. *Plant Science*, **168:** 607-613.

Feng, W., Kita, D., Peaucelle, A., Cartwright, H.N., Doan, V., Duan, Q. 2018. The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Current Biology*, **28:** 666-675.

Giri, S., Saha, T., Maiti, M. 2024. Seed priming: A strategy to mitigate flooding stress in pulses. *International Journal of Environment and Climate Change*, **14:**42-55.

Hamayun, M., Hussain, A., Khan, S.A., Irshad, M., Khan, A.L., Waqas, M., Shahzad, R., Iqbal, A., Ullah, N., Rehman, G., Kim, H.Y., Lee, I.J. 2015. Kinetin modulates physiohormonal attributes and isoflavone contents of soybean grown under salinity stress. *Frontiers in Plant Science*, **6:** 377.

Heath, R.L., Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125:** 189-198.

Islam, M.M., Ahmed, M., Mahalder, D. 2005. In *vitro* callus induction and plant regeneration in seed explants of rice (*Oryza sativa* L.). *In Vitro Cell and Developmental Biology Plant*. **41:** 72-75.

Kong, D., Ju, C., Parihar, A., Kim, S., Cho, D., Kwak, J.M. 2015. Arabidopsis glutamate receptor homolog 3.5 modulates cytosolic Ca²? level to counteract effect of abscisic acid in seed germination. *Plant Physiology*, **167:** 1630-1642.

Lutts, S., Almansouri, M., Kinet, J.M. 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Science*, **167:** 9-18.

Lutts, S., Paolo, B., Lukasz, W.S.K.S., Robert, P. 2016. Seed priming: new comprehensive approaches for an old empirical technique. In: New *Challenges in Seed Biology: Basic and Translational Research Driving Seed Technology,* S. Araujo (ed.), pp. 1-46, IntechOpen, Rijeka, Croatia.

Marschner, H. 1995. Function of mineral nutrients. In: *Mineral Nutrition of Higher Plants,* H. Marschner (ed.), pp. 229-299, Academic Press, London, UK.

Mamdouh, D., Smetanska, I. 2022. Optimization of callus and cell suspension cultures of *Lycium schweinfurthii* for improved production of phenolics, flavonoids, and antioxidant activity. *Horticulturae*, **8:** 394.

Naik, P.S., Widholm, J.M. 1993. Comparison of tissue culture and whole plant responses to salinity in potato. *Plant Cell, Tissue and Organ Culture*, **33:** 273-280.

Niu, L., Yu, J., Liao, W., Yu, J., Zhang, M., Dawuda, M.M. 2017. Calcium and calmodulin are involved in nitric oxide-induced adventitious rooting of cucumber under simulated osmotic stress. *Frontiers in Plant Science*, **8:** 1684.

Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., Balestrazzi, A. 2015. Seed priming: State of the art and new perspectives. *Plant Cell Reports*, **34:** 1281-1293.

Pasternak, T.P., Steinmacher, D. 2024. Plant growth regulation in cell and tissue culture. *In Vitro Plants*. **13:** 327-345.

Queiros, F., Fidalgo, F., Santos, I., Salema, R. 2007. In vitro selection of salt tolerant cell lines in *Solanum tuberosum* L. *Biologia Plantarum*, **51:** 728-734.

Rahman, A., Nahar, K., Hasanuzzaman, M., Fujita, M. 2016. Calcium supplementation improves Na?/K? ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. *Frontiers in Plant Science*, **7:** 609.

Sairam, P.K., Srivastava, G.C. 2002. Changes in antioxidant activity in subcellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Science*, **162:** 897-904.

Sharma, V., Ramawat, K.G. 2013. Salinity-induced modulation of growth and antioxidant activity in the callus cultures of miswak (*Salvadora persica*). *BioTechnology*, **3:** 11-17.

Singh, R.B., Vivekanand, P., Rao, R., Sengar, S. 2023. Study of salinity induced oxidative stress and antioxidant responses in callus cultures of sugarcane. *Ecological Genetics and Genomics*, **26:** 2405-9854.

Skoog, F., Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissue cultures in *vitro*. *Symposium of the Society for Experimental Biology*, **11:** 118-131.

Tamimi, S., Othman, H. 2021. Callus induction and regeneration from germinating seeds of wheat (*Triticum aestivum* L.). *Sains Malaysiana*, **50:** 889-896.

Wang, W., Chen, Q., Hussain, S., Mei, J., Dong, H., Peng, S., Huang, J., Cui, K., Nie, L. 2016. Pre-sowing seed treatments in direct-seeded early rice: Consequences for emergence, seedling growth and associated metabolic events under chilling stress. *Scientific Reports*, **6:** 19637.

Zhang, H.J., Zhang, N., Yang, R.C., Wang, L., Sun, Q.Q., Li, D.B., Cao, Y.Y., Weeda, S., Zhao, B., Ren, S., Guo, Y.D. 2014. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research*, **57:** 269-279.

Zhang, Z., Fan, J., Wu, J., Zhang, L., Wang, J., Zhang, B., Wang-Pruski, G. 2020. Alleviating effect of silicon on melon seed germination under autotoxicity stress. *Ecotoxicology and Environmental Safety*, **188:** 109901.