BioRisk 17: 227–240 (2022)
doi: 10.3897/biorisk.17.77465
https://biorisk.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
BioRisk

# Influence of proline and methyl jasmonate priming on in vitro seed germination and seedling development of *Chelidonium majus* L

Iva Doycheva[1]

1 *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Department of Plant and Fungal Diversity and Resources, 23 Acad. G. Bonchev str., 1113 Sofia, Bulgaria*

Corresponding author: Iva Doycheva (idoycheva@gmail.com)

Academic editor: Kalina Danova | Received 1 November 2021 | Accepted 14 December 2021 | Published 21 April 2022

**Citation:** Doycheva I (2022) Influence of proline and methyl jasmonate priming on in vitro seed germination and seedling development of *Chelidonium majus* L. In: Chankova S, Peneva V, Metcheva R, Beltcheva M, Vassilev K, Radeva G, Danova K (Eds) Current trends of ecology. BioRisk 17: 227–240. https://doi.org/10.3897/biorisk.17.77465

## Abstract
Drought, salinization and heavy metal pollution of soils are main stress factors with an increasing impact on the deterioration of soil quality, yield and crop quality. Seed priming shows good results in improving seed germination, seedling growth and plant development. Proline (Pro) and metyl jasmonate (MeJA) show stimulating activity and help plants overcome stress. The study investigated the effect of Pro, MeJA and hydropriming on seeds sown on water agar supplemented with different concentrations of heavy metals (Cd, Pb, Zn) (HM), NaCl or Polyethylene glycol 6000 (PEG 6000). *Chelidonium majus* is a medicinal species which is grown as a crop in some parts of Europe. It is an ingredient in some remedies and is becoming an increasingly popular object of research regarding its biological activities. The low concentrations of all heavy metals applied increased the germination of all variants of seeds – control, hydroprimed and those which were Pro and MeJA primed. Seed priming with Pro and MeJA promoted high germination percentage of seeds germinated on water agar with NaCl. PEG 6000 at its higher concentration (5%) slightly increased the seed germination of all variants. The growth of roots and hypocotyls was inhibited by HM and NaCl. However, PEG 6000 slightly influenced their growth.

## Keywords
Abiotic stress, drought, elicitors, Greater celandine, heavy metals, hydropriming, salt stress

## Introduction

Drought, salt and heavy metal stress are among the main abiotic stresses which lead to biochemical, molecular, and morpho-physiological alterations, and as a result influence plant growth, development and metabolism (Bhanuprakash and Yogeesha 2016). Anthropogenic activities such as smelting, mining, overuse of pesticides and fertilizers, sewage sludge disposal directly pollute and worsen the environmental conditions through accumulation of heavy metals. Additionally, industrialization, environmental pollution and unwise water utilization lead to climate changes and the increasing consequences of this – drought and soil salinization (Ullah et al. 2018; Ghori et al. 2019). Drought, salinization and heavy metal pollution of soils have an adverse impact on the deterioration of yield, crop and soil quality and as an extreme case result in the loss of arable land. These stress factors decrease seed germination percentage, uniformity and speed of seed germination, deteriorate seedling growth, reduce root and shoot length. As these problems increase, the quest to create new varieties, ones which could be tolerant to the aforementioned factors, is becoming more and more popular. Such a technique is seed priming, which shows good results in improving seed germination, seedling growth and plant development, and in the process of crop establishment in general. Seed priming leads to partial hydration of seeds which have been soaked in a certain solution but without inducing radicle protrusion (Chen and Arora 2011). There are different methods of priming depending on the pre-sowing conditions in which seeds are set: hydro-, osmo-, halo-, thermopriming, priming using plant growth regulators, matrix priming, etc. Seed priming alleviates stress effects and promotes stress tolerance of crops which could allow them to grow even in adverse conditions. Seed priming reduces germination time and increases germination percentage and plant hardiness even under unfavorable conditions. Moreover, the benefit of this method is the low cost, easy applicability and effectiveness (Bhanuprakash and Yogeesha 2016). Seed germination and early stages of seedling growth are the most vulnerable phases in adverse conditions which could further slow down germination onset, and uniformity and crop growth could be poor with low production (Bhanuprakash and Yogeesha 2016). Seed priming seems to be one of the methods which could improve seed germination and plant growth performance under drought, salt and heavy metal stress (Roychoudhury et al. 2016). In hormonpriming, chemopriming, plant growth regulators and chemicals are used such as proline and methyl jasmonate (MeJA). They both are biosynthesized by plants in order to overcome stress. The amino acid proline (Pro) plays a crucial role in alleviating abiotic stress as an osmoprotectant, a metal chelator and an antioxidant through its accumulation in plants (Hayat et al. 2012; Aslam et al. 2017). Exogenous application of Pro on different plant species contributes to overcoming stress factors. Pro stimulated seed germination of *A. thaliana* (Hare et al. 2003) and *Triticum aestivum* seeds pre-soaked with Pro solutions influenced plant growth under drought stress (Kamran et al. 2009). Pro improved seed germination and seedling growth of rice under salt stress (Roy et al. 1993). The exogenous treatment of *Solanum nigrum* with Pro increased its tolerance as hyperaccumulator to cadmium (Xu et al. 2009). The exogenous usage of plant growth regulators has become recently more popular in order to increase plant tolerance to abiotic stress

(Farooq et al. 2016). MeJA is a phytohormone which acts as a signal in plants which are submitted to abiotic and biotic stress (Fujita et al. 2006). MeJA alleviated the inhibitory effects of Cd on *Solanum nigrum* and *Glycine max* (Keramat et al. 2009; Yan et al. 2015). It mitigated the adverse effects of drought on seed germination and seedling growth of *Oryza sativa* (Sheteiwy et al. 2018) and the effects of salinity stress on primed lemon seeds (Asadi and Jalilian 2021). Seed priming with MeJA increased germination percentage and early seedling growth of *Solanum melongena* (Ali et al. 2019).

*Chelidonium majus* L. is a medicinal species which has promising pharmacological properties and attracts scientific attention which leads to increasing research. The species is an ingredient of some conventional herbal remedies. Moreover, it is used as a homeopathic drug administered in cases of liver disorders and cancer in humans. The species has anti-inflammatory, anti-tumor, anti-microbial and anti-viral properties (Biswas and Khuda-Bukhsh 2002). *Ch. majus* is cultivated as a crop in several countries in Central Europe (Zielinska et al. 2018). As a crop culture the species will face the challenges of an increasingly changing and more unpleasant environment. The changing environment and increasing pharmacological interest in the species require that the species' hardiness to the growing negative influence of abiotic factors be studied. The aim of the study was to ascertain if seed priming with Pro and MeJA mitigates the adverse effect of heavy metals, salt and drought on seed germination and seedling growth of *Ch. majus* in water agar medium.

## Materials

The seeds of *Ch. majus* originated from plants of the species grown in a native habitat in the village of Mramor, near Sofia, Bulgaria (42.7885°N, 23.2794°E). Two-year-old seeds in good condition with uniform size were subjected to three priming treatments: hydro-priming (priming with distilled water), Pro-priming (with 30mM proline), MeJA-priming (with 1 mg/l MeJA). Seed priming was done by soaking the seeds in distilled water, 30 mM Pro or 1 mg/l MeJA solutions for 24 hours at room temperature and then they were dried under a laminar air flow cabinet for an hour. The seeds were soaked in 70% ethanol for 2 minutes as a first step. Then they were sterilized in 0.1% $HgCl_2$ for 2 minutes and then washed three times in sterile distilled water. After that the seeds were cleansed for 10 minutes in NaClO (chlorine < 2.5%) half diluted with sterile distilled water. As a last step the seeds were triple rinsed with sterile distilled water (Doycheva in press). Sterilized seeds were sown in water agar medium. The medium consisted of distilled water solidified with 7 g/l plant agar (Duchefa, NL) and autoclaved at 121 °C for 20 minutes. The autoclaved water agar (5.5 pH) was supplemented with different compounds: $Pb(NO_3)_2$, $ZnSO_4 \cdot 7H_2O$, $CdCl_2 \cdot 2½H_2O$, which were added to create heavy metal stress. The chemical compounds were added in order to obtain certain concentrations of metal ions: 100; 150; 250 mg/l $Pb^{2+}$ or $Zn^{2+}$ ions and 1; 5 and 10 mg/l $Cd^{2+}$. NaCl and Polyethylene glycol 6000 (PEG 6000) were supplemented to the distilled water before adding the plant agar, and then the medium was autoclaved. The following concentrations were applied: 50, 100, 150 mM NaCl, and 1% and 5% PEG 6000. The control variants were hydro-primed seeds sown on water agar (WA). The seeds were sown in sterilized plastic petri dishes (90 mm in diameter) with hardened medium supplemented with one of the various compounds. The petri dishes were enveloped with Parafilm. Seeds were put in the dark, first at 8±2 °C for 7 days and then at 23±2 °C for 2 weeks. After that seeds were set at 23±2 °C with photoperiod of 16 h light/8 h dark (Doycheva in press).

Each treatment had 3 replications with 20 seeds in each. Radicle emergence was considered a sign of germination. Pro primed seedlings grown on WA + 5% PEG 6000 were contaminated, that is why the values for root and hypocotyl length are absent.

Germination percentage, mean root and hypocotyl length, tolerance index and phytotoxicity percentage were measured to determine the significance of priming on *Ch. majus* seeds to mitigate the adverse effects of stress factors (Rasafi et al. 2016). The closer a certain value of the tolerance index was to 1, the more phytotoxic the studied compound was.

Results were shown as mean values of the three replications with ± standard deviation (SD). Statistical significance was evaluated with Student's t-test at $p \leq .05$. Values with different letters in the table and figures were significantly different. The statistical analyses were done using SigmaPlot v. 14.0

## Results

### Germination percentage, root and hypocotyl length of Pro and MeJA primed seeds germinated on WA supplemented with HM

Seed priming with Pro and MeJA slightly increased the germination compared to that of the seeds sown on water agar without HMs. The addition of Pb2+ to the medium raised the germination percentages in all sown seeds, no matter if they were primed with solutions of the elicitors (Pro and MeJA) or if they were just hydroprimed (Fig. 1). Surprisingly, higher Pb2+ concentrations resulted in higher germination percentages.

Seeds primed with MeJA had the highest germination percentages in all applied concentrations of Zn2+ (Fig. 2). In contrast, the increase in Zn2+ concentrations in the medium led to a decrease in the germination percentage of the hydroprimed seeds and those primed with Pro. However, the germination percentage of all primed seeds sown on WA + HM was higher than that of the Control (Fig. 2).

Cd2+ had the opposite effect to that of Zn2+ on the MeJA primed seeds. The higher Cd2+ concentrations were, the higher the reduction in the germination percentage of MeJA pre-soaked seeds was (Fig. 3). Thus, the germination percentage of the seeds primed with MeJA was highest at the lowest Cd2+ concentration (1 mg/l) and dropped to 64.71% at the highest Cd2+ concentration. The same trend was observed in the germination of Pro primed and hydroprimed seeds (Fig. 3).

HMs availability in the medium reduced the root and hypocotyl length (Table 1). That was most significantly observed in the seedlings grown in medium with Pb2+ and

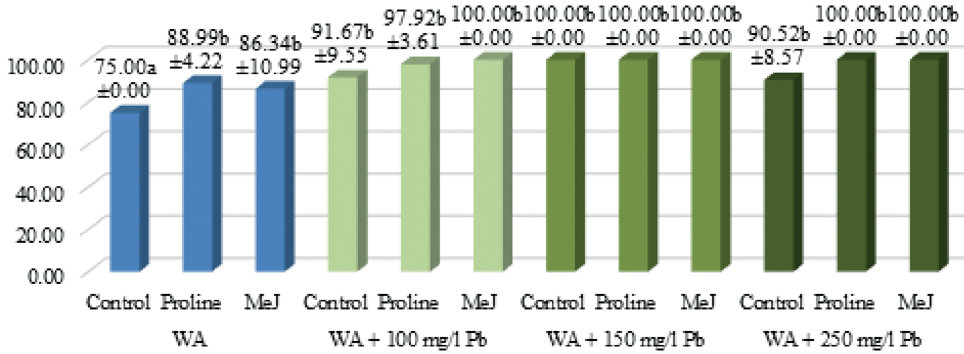


**Figure 1.** Germination percentage (%) of Pro and MeJA primed seeds germinated on WA + Pb.

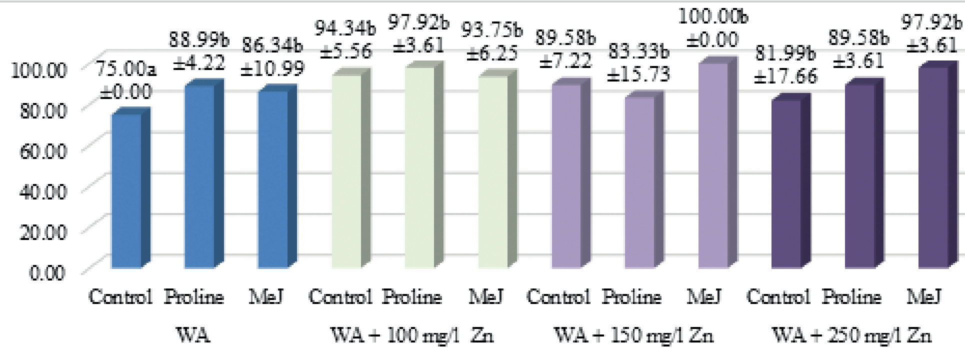


**Figure 2.** Germination percentage (%) of Pro and MeJA primed seeds germinated on WA + Zn.

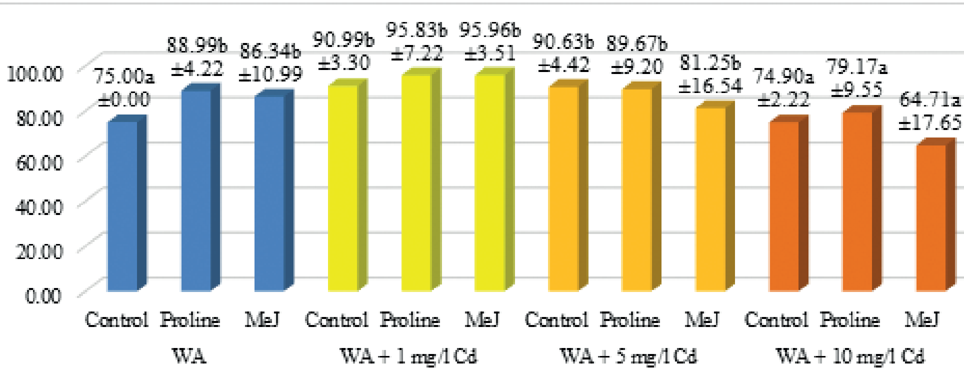


**Figure 3.** Germination percentage (%) of Pro and MeJA primed seeds germinated on WA+Cd.

Zn2+ and less so in those grown in the presence of Cd2+. The radicles of seedlings on media with Pb2+ and Zn2+ only protruded the seed coat, turned black and perished. Seed priming didn't contribute to overcoming the inhibitory influence of the HM on the growth and development of the seedlings.

**Table 1.** The effect of HMs, NaCl and PEG 6000 on mean hypocotyl and root length of *Ch. majus* seedlings.

| Media | Mean hypocotyl length (mm) | | | Mean root length (mm) | | |
|---|---|---|---|---|---|---|
| | Control | Pro | MeJA | $H_2O$ | Pro | MeJA |
| WA | 29.65a±1.14 | 31.01a±2.85 | 30.47a±1.78 | 23.33a±2.23 | 21.56a±0.64 | 21.13a±4.05 |
| $Pb^{2+}$ 100 mg/l | 19.53b±1.69 | 17.94b±2.13 | 17.76b±1.47 | - | - | - |
| $Pb^{2+}$ 150 mg/l | 14.41c±1.06 | 13.05c±1.18 | 14.21c±1.85 | - | - | - |
| $Pb^{2+}$ 250 mg/l | 11.72c±1.04 | 11.64c±1.29 | 11.51c±1.37 | - | - | - |
| $Zn^{2+}$ 100 mg/l | 8.89c±1.63 | 9.60c±2.41 | 12.54c±2.30 | - | - | - |
| $Zn^{2+}$ 150 mg/l | 7.40d±1.44 | 6.62d±0.79 | 7.39d±1.50 | - | - | - |
| $Zn^{2+}$ 250 mg/l | 6.34d±0.98 | 5.40d±0.91 | 6.65d±0.79 | - | - | - |
| $Cd^{2+}$ 1 mg/l | 19.74b±2.06 | 17.02b±0.47 | 13.18c±2.78 | 4.03b±0.24 | 3.54b±0.28 | 3.33b±0.53 |
| $Cd^{2+}$ 5 mg/l | 11.23c±2.21 | 11.86c±2.67 | 11.36c±2.44 | 1.49c±0.22 | 1.63c±0.04 | 1.44c±0.31 |
| $Cd^{2+}$ 10 mg/l | 12.56c±1.26 | 8.17c±2.33 | 11.70c±2.47 | 1.05c±0.06 | 1.00c±0.00 | 1.00c±0.00 |
| WA + 50 mM NaCl | 11.68b±2.52 | 10.56b±0.13 | 12.37b±3.15 | 5.70b±1.44 | 6.98b±0.83 | 7.23b±0.93 |
| WA + 100 mM NaCl | 5.39c±1.51 | 3.46c±0.47 | 5.18c±0.33 | 1.00c±0.00 | 1.00c±0.00 | 1.00c±0.00 |
| WA + 150 mM NaCl | 3.83c±1.27 | 3.45c±0.71 | 3.17c±0.31 | 0.00c±0.00 | 1.00c±0.00 | 1.00c±0.00 |
| WA + 1% PEG 6000 | 27.25a±5.87 | 28.21a±3.63 | 23.41a±2.77 | 21.36a±6.30 | 27.32a±13.95 | 18.10a±7.88 |
| WA + 5% PEG 6000 | 22.27a±4.92 | N/A | 21.08a±0.65 | 17.73a±6.17 | N/A | 20.86a±0.34 |

**Table 2.** The effect of HM, NaCl and PEG 6000 on tolerance index and phytotoxicity percentage of *Ch. majus*.

| Media | Tolerance index | | | Phytotoxicity percentage (%) | | |
|---|---|---|---|---|---|---|
| | $H_2O$ | Pro | MeJA | $H_2O$ | Pro | MeJA |
| WA | 100.00d±0.00 | 93.06d±12.74 | 90.19d±8.77 | 0.00f±0.00 | 0.07f±0.13 | 0.1f±0.09 |
| WA + 50 mM NaCl | 22.79bc±9.29 | 28.34c±0.63 | 32.98c±0.26 | 0.77c±0.09 | 0.72c±0.01 | 0.67d±0.00 |
| WA + 100 mM NaCl | 4.31a±0.41 | 4.31a±0.41 | 4.31a±0.41 | 0.96b±0.00 | 0.96b±0.00 | 0.96b±0.00 |
| WA + 150 mM NaCl | reduced | 4.31a±0.41 | 4.31a±0.41 | reduced | 0.96b±0.00 | 0.96b±0.00 |
| WA + 1% PEG 6000 | 103.99d±11.39 | 144.06d±33.78 | 68.92d±38.13 | -0.04f±0.11 | -0.44f±0.34 | 0.31f±0.38 |
| WA + 5% PEG 6000 | 77.63d±33.86 | N/A | 89.90d±10.05 | 0.22f±0.34 | N/A | 0.10f±0.10 |
| $Cd^{2+}$ 1 mg/l | 17.42b±2.67 | 15.13b±0.26 | 13.81b±1.76 | 0.83a±0.03 | 0.85a±0.00 | 0.86a±0.02 |
| $Cd^{2+}$ 5 mg/l | 6.36a±0.33 | 6.96a±0.84 | 6.96a±0.55 | 0.94c±0.00 | 0.93c±0.01 | 0.93c±0.01 |
| $Cd^{2+}$ 10 mg/l | 4.52a±0.71 | 4.31a±0.41 | 4.31a±0.41 | 0.95b±0.01 | 0.96b±0.00 | 0.96b±0.00 |

Pb2+ and Zn2+ were extremely phytotoxic for seedling development. Cd2+ also had very high phytotoxicity, but the roots didn't turn black and stop their growth at 1 mm at the lowest Cd2+ concentration. The tolerance index of the Pro and MeJA primed seeds was higher than that of the water pre-soaked seeds at 1 mg/l Cd2+. However, the tolerance index sharply dropped at 5 and 10 mg/l Cd2+ and the indices of elicitor primed seeds and hydroprimed seeds were almost equal (Table 2).

## Effect of Pro and MeJA priming on germination and seedling growth in agar medium supplemented with NaCl

NaCl significantly reduced the germination of the hydroprimed seeds. Pro and MeJA enhanced the germination of seeds sown in the media with NaCl compared to the germination percentage of the hydroprimed seeds on the same media. However, the higher the NaCl concentration was, the lower the alleviating effect of Pro and MeJA was (Fig. 4).

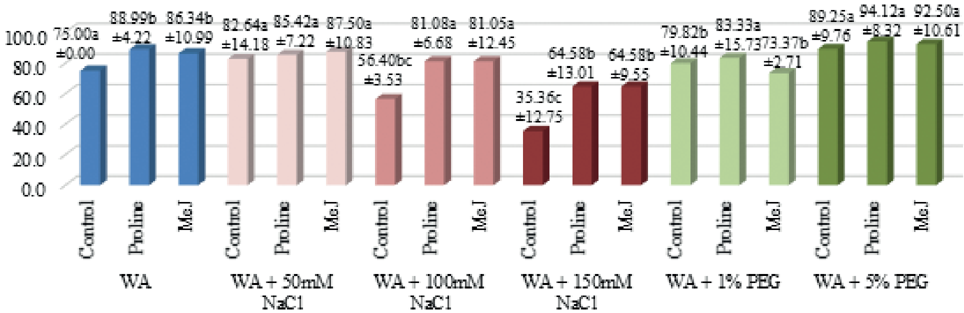


**Figure 4.** Germination percentage (%) of Pro and MeJA primed seeds germinated on WA+NaCl and WA+PEG.

NaCl also significantly reduced the length of roots and hypocotyls (Table 1). Seed priming with the elicitors didn't promote the reduction of the unfavorable influence of NaCl on root and hypocotyl growth. *Ch. majus* seeds had very low tolerance to NaCl at the higher applied concentrations. Above 50 mM NaCl the values of phytotoxicity and tolerance index were the same for the hydroprimed seeds and those primed with the elicitors (Table 2). However, at 150 mM NaCl the roots of the hydroprimed seeds were absolutely reduced.

### Effect of Pro and MeJA priming on seed germination and seedling growth under drought conditions induced by PEG 6000 supplementation

Seed priming didn't influence significantly the germination percentage of seeds in medium supplemented with PEG 6000. The percentages of primed germinated seeds were close to or much higher than the Control value. The germination was more stimulated at 5% PEG 6000 (Fig. 4). The root length of Pro primed seeds on WA + 1% PEG 6000 was significantly higher than that of the Control (Table 1). The values of the other variants were close to that of the Control. PEG 6000 slightly reduced the length of hypocotyls and its effect enhanced with the increase of PEG concentration (Table 1). The Pro- and hydroprimed seeds had high tolerance to the lower concentration of PEG 6000 and there was no phytotoxicity (Table 2). The positive influence of MeJA priming occurred at the higher PEG concentration.

## Discussion

The process of seed sprouting and especially the early stages of seedling growth are the most vulnerable stages of plant development. The stress conditions can reduce germination and retard its onset, which results in weak plant development and low productivity (Bhanuprakash and Yogeesha 2016). Plants acclimatize to the unfavorable environmental conditions through activation of complex internal mechanisms with the final aim to respond to external factors in the most optimal way and to survive the stress conditions (Hossain et al. 2014). Thus, Pro accumulation occurs when plants are subjected to salt, drought and heavy metal stress and can be provoked by its exogenous application (Hayat et al. 2012). Similarly, exogenous application of MeJA elevates endogenous levels of Pro and MeJA which provides protection of plants under abiotic stress (Yan et al. 2015). Exogenous applications of MeJA – increased JA content and biosynthesis of secondary metabolites, which have a defensive role in the alleviation of abiotic and biotic stress in plants (Dar et al. 2015; Farooq et al. 2016).

The results showed that Pro and MeJA priming stimulated the seed germination on WA.

Overall, the germination of seeds on WA supplemented with Pb was high, which probably hid the Pro and MeJA effect. According to Parera and Cantliffe (1994) the beneficial influence of priming is more visible at adverse conditions than at favorable ones. The results of the study suggested that Pb2+ did not inhibit the germination of *Ch. majus* seeds. Yang et al. (2010) observed that the germination percentage of wheat seeds was increased after treatment with 1mM Pb. The seeds on WA with Zn2+ also had high germination percentage. However, the increase of Zn2+ concentration promoted MeJA mitigation of the inhibitory effect of Zn2+. On the other hand, the higher Cd2+ concentration applied reduced the protective influence of MeJA.

In regard to Pro priming, the strengthening of the stress factor influence (the increase of HMs concentration) led to the reduction of the Pro effect. In the current study, the germination percentage of seeds on WA supplemented with HMs was high but HMs greatly inhibited the seedling development. This might be caused by the impermeability of the seed coat to some HM and the role of the seed coat for seed tolerance (Li et al. 2005; Akinci and Akinci 2010). The fact that seeds germinated at high HM concentrations but seedling growth was retarded at low concentrations indicated exactly that (Akinci and Akinci 2010). According to the research of Yang et al (2010) though the low Pb concentrations improved the germination of wheat seeds its higher concentrations inhibited the further growth of the seedlings. It was reported that HMs are more toxic to seedling growth than to their germination in *A. thaliana*, too (Li et al. 2005). HMs strongly inhibited the roots which could be explained with the fact that roots are the first contact organ with the stress factor in growth medium (Andresen and Küpper 2013). NaCl significantly inhibited the germination percentage because of perturbed water imbibition, hyperosmotic stress and aggravated stored food mobilization (Kalaji and Pietkiewicz 1993; Johnson and Puthur 2021). However, Pro and MeJA promoted higher percentage of germination. The treatment with NaCl revealed the beneficial effect of priming with the elicitors, whereas hydropriming did not decrease the adverse effect of salt stress. Pro and MeJA priming promoted the metabolic processes which occurred during the pre-germination period (Paparella et al. 2015). The negative effect of NaCl on seedling growth is because of high salt accumulation and osmotic stress, cytotoxic ion gathering, increased transpiration because of impaired stomatal closure. Seed germination and seedling growth were not influenced negatively by drought stress to a great extent and MeJA effect was stronger at higher PEG concentration. MeJA alleviating effect was observed in the osmotic stress in *Oryza sativa* through a change in the physiology of the seedlings (Sheteiwy et al. 2018). Pro and MeJA degree of influence depend on the concentration applied, the stage of plant development and the way of application on plants (in the medium or leaf spraying) (Ashraf and Foolad 2007). Further studies are required in order to elucidate the influence of all previously mentioned factors on the overall mitigating effect of Pro and MeJA on stress factors.

## Conclusion

The influence of Pro and MeJA priming on seed germination depended on the type of the stress factor applied. Thus, HM and PEG 6000 did not inhibit seed germination or inhibited it to a small extent, and Pro and MeJA priming effect remained hidden. However, NaCl decreased the germination percentage, – but elicitor priming compensated the adverse effect of NaCl. Seedling growth was influenced by the concentration and type of stress agent and by the priming treatment. Thus, Zn reduced seedling growth to the greatest extent. And Pro priming increased root length at 1% PEG 6000.

## Acknowledgements

This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Project КП-06-М26/4 from 01.12.2018).

## References

Akinci IE, Akinci S (2010) Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.). African Journal of Biotechnology 9: 4589–4594. http://www.academicjournals.org/AJB

Ali M, Hayat S, Ahmad H, Ghani MI, Amin B, Atif MJ, Cheng Z (2019) Priming of *Solanum melongena* L. Seeds Enhances Germination, Alters Antioxidant Enzymes, Modulates ROS, and Improves Early Seedling Growth: Indicating Aqueous Garlic Extract as Seed-Priming Bio-Stimulant for Eggplant Production. Applied Sciences (Basel, Switzerland) 9(11): e2203. https://doi.org/10.3390/app9112203

Andresen E, Küpper H (2013) Cadmium toxicity in plants. In: Sigel A, Sigel H, Sigel RKO (Eds) Cadmium: From Toxicity to Essentiality. Metal Ions in Life Sciences. Springer, Dordrecht, 395–409. https://doi.org/10.1007/978-94-007-5179-8_13

Asadi N, Jalilian S (2021) The effect of methyl jasmonate on the germination of lemon seeds under the influence of salinity stress. Central Asian Journal of Environmental Science and Technology Innovation 3: 119–128. https://doi.org/10.22034/CAJESTI.2021.03.03

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59(2): 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Aslam M, Saeed MS, Sattar S, Sajad S, Sajjad M, Adnan M, Iqbal M, Sharif MT (2017) Specific role of proline against heavy metals toxicity in plants. Internationl Journal of Pure and Applied Bioscience 5(6): 27–34. https://doi.org/10.18782/2320-7051.6032

Bhanuprakash K, Yogeesha HS (2016) Seed Priming for Abiotic Stress Tolerance: An Overview. In: Rao NKS, Shivashankara KS, Laxman RH (Eds) Abiotic Stress Physiology of Horticultural Crops. Springer (India), 103–117. https://doi.org/10.1007/978-81-322-2725-0_6

Biswas SJ, Khuda-Bukhsh AR (2002) Effect of a homeopathic drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice. BMC Complementary Medicine and Therapies 2(1): e4. https://doi.org/10.1186/1472-6882-2-4

Chen K, Arora R (2011) Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in spinach (Spinacia oleracea). Plant Science 180(2): 212–220. https://doi.org/10.1016/j.plantsci.2010.08.007

Dar TA, Uddin M, Khan MMA, Hakeem KR, Jaleel H (2015) Jasmonates counter plant stress: A review. Environmental and Experimental Botany 115: 49–57. https://doi.org/10.1016/j.envexpbot.2015.02.010

Doycheva I (in press) The effect of some heavy metals (Cd, Cu, Pb, Zn) and substrates on *Chelidonium majus* L. seed germination and seedling growth. Ecologia Balkanica.

Farooq MA, Gill RA, Islam F, Ali B, Liu H, Xu J, He S, Zhou W (2016) Methyl Jasmonate Regulates Antioxidant Defense and Suppresses Arsenic Uptake in *Brassica napus* L. Frontiers in Plant Science 7: e468. https://doi.org/10.3389/fpls.2016.00468

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: A current view from the points of convergence in the stress signaling networks. Current Opinion in Plant Biology 9(4): 436–442. https://doi.org/10.1016/j.pbi.2006.05.014

Ghori NH, Ghori T, Hayat MQ, Imadi SR, Gul A, Altay V, Ozturk M (2019) Heavy metal stress and responses in plants. International Journal of Environmental Science and Technology 16(3): 1807–1828. https://doi.org/10.1007/s13762-019-02215-8

Hare PD, Cress WA, van Staden J (2003) A regulatory role for proline metabolism in stimulating *Arabidopsis thaliana* seed germination. Plant Growth Regulation 39(1): 41–50. https://doi.org/10.1023/A:1021835902351

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: A review. Plant Signaling & Behavior 7(11): 1456–1466. https://doi.org/10.4161/psb.21949

Hossain M, Hoque M, Burritt D, Fujita M (2014) Proline Protects Plants Against Abiotic Oxidative Stress: Biochemical and Molecular Mechanisms. In: Ahmad P (Ed.) Oxidative Damage to Plants. Antioxidant Networks and Signaling. Academic Press, 477–522. https://doi.org/10.1016/B978-0-12-799963-0.00016-2

Johnson R, Puthur JT (2021) Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress. Plant Physiology and Biochemistry 162: 247–257. https://doi.org/10.1016/j.plaphy.2021.02.034

Kalaji MH, Pietkiewicz S (1993) Salinity effects on plant growth and other physiological processes. Acta Physiologiae Plantarum 15: 89–124. https://doi.org/10.1016/j.plaphy.2021.02.034

Kamran M, Shahbaz M, Ashraf M, Akram NA (2009) Alleviation of drought- induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as a pre-sowing seed treatment. Pakistan Journal of Botany 41: 621–632.

Keramat B, Kalantari KM, Arvin MJ (2009) Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (*Glycine max* L.). African Journal of Microbiological Research 3: 240–244. http://www.academicjournals.org/ajmr

Li W, Khan MA, Yamaguchi S, Kamiya Y (2005) Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana.* Plant Growth Regulation 46(1): 45–50. https://doi.org/10.1007/s10725-005-6324-2

Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A (2015) Seed priming: State of the art and new perspectives. Plant Cell Reports 34(8): 1281–1293. https://doi.org/10.1007/s00299-015-1784-y

Parera CA, Cantliffe DJ (1994) Presowing seed priming. Horticultural Reviews 16: 109–114. https://doi.org/10.1002/9780470650561.ch4

Rasafi T, Nouri M, Bouda S, Haddioui A (2016) The effect of Cd, Zn and Fe on seed germination and early seedling growth of wheat and bean. Ekologia (Bratislava) 35(3): 213–223. https://doi.org/10.1515/eko-2016-0017

Roy D, Basu N, Bhunia A, Banerjee SK (1993) Counteraction of exogenous-proline with NaCl in salt-sensitive cultivar of rice. Biologia Plantarum 35(1): 69–72. https://doi.org/10.1007/BF02921122

Roychoudhury A, Ghosh S, Paul S, Mazumdar S, Das G, Das S (2016) Pre-treatment of seeds with salicylic acid attenuates cadmium chloride-induced oxidative damages in the seedlings of mungbean (*Vigna radiata* L. Wilczek). Acta Physiologiae Plantarum 38(1): e11. https://doi.org/10.1007/s11738-015-2027-0

Sheteiwy MS, Gong D, Gao Y, Pan R, Hu J, Guan Y (2018) Priming with methyl jasmonate alleviates polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile. Environmental and Experimental Botany 153: 236–248. https://doi.org/10.1016/j.envexpbot.2018.06.001

Ullah A, Manghwar H, Shaban M, Khan AH, Akbar A, Ali U, Ali E, Fahad S (2018) Phytohormones enhanced drought tolerance in plants: A coping strategy. Environmental Science and Pollution Research International 25(33): 33103–33118. https://doi.org/10.1007/s11356-018-3364-5

Xu J, Yin H, Li X (2009) Protective effects of Proline against cadmium toxicity in micropropagated hyperaccumulator, *Solanum nigrum* L. Plant Cell Reports 28(2): 325–333. https://doi.org/10.1007/s00299-008-0643-5

Yan Z, Zhang W, Chen J, Li X (2015) Methyl jasmonate alleviates cadmium toxicity in *Solanum nigrum* by regulating metal uptake and antioxidative capacity. Biologia Plantarum 59(2): 373–381. https://doi.org/10.1007/s10535-015-0491-4

Yang Y, Wei X, Lu J, You J, Wang W, Shi R (2010) Lead-induced phytotoxicity mechanism involved in seed germination and seedling growth of wheat (*Triticum aestivum* L.). Ecotoxicology and Environmental Safety 73(8): 1982–1987. https://doi.org/10.1016/j.ecoenv.2010.08.041

Zielinska S, Wójciak-Kosior M, Płachno BJ, Sowa I, Włodarczyk M, Matkowski A (2018) Quaternary alkaloids in *Chelidonium majus* in vitro cultures. Industrial Crops and Products 123: 17–24. https://doi.org/10.1016/j.indcrop.2018.06.062

## Supplementary material 1

**Figure S1**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean hypocotyl length (mm) of seedlings grew on WA+NaCl and WA + PEG 6000.



Link: https://doi.org/10.3897/biorisk.17.77465.suppl1

## Supplementary material 2

**Figure S2**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean hypocotyl length (mm) of seedlings grown on WA+Cd (mg/l).



Link: https://doi.org/10.3897/biorisk.17.77465.suppl2

## Supplementary material 3

**Figure S3**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean hypocotyl length (mm) of seedlings grown on WA+Pb (mg/l).



Link: https://doi.org/10.3897/biorisk.17.77465.suppl3

## Supplementary material 4

**Figure S4**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean hypocotyl length (mm) of seedlings grown on WA+Zn (mg/l).



Link: https://doi.org/10.3897/biorisk.17.77465.suppl4

## Supplementary material 5

**Figure S5**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean root length (mm) of seedlings grew on WA+NaCl and WA + PEG 6000.



Link: https://doi.org/10.3897/biorisk.17.77465.suppl5

## Supplementary material 6

**Figure S6**

Authors: Iva Doycheva

Data type: docx file

Explanation note: Mean root length (mm) of seedlings grown on WA+Cd (mg/l).



Link: https://doi.org/10.3897/biorisk.17.77465.suppl6