Effect of methyl jasmonate seed treatment on physiological processes in wheat plants under conditions of increased zinc content in substrate

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Abstract. The effect of methyl jasmonate (MeJA, 10 μM) seed treatment on seed germination, growth and some parameters of photosynthesis and water metabolism in wheat (*Triticum aestivum* L.) plants of the Zlata variety grown at optimal (5 mg/kg) or excessive (46 mg/kg) zinc content in the substrate was studied. It was shown that elevated zinc concentrations inhibited shoot growth and led to a decrease both in chlorophyll content and transpiration intensity. MeJA seed treatment stimulated germination, but slightly inhibited plant growth during the tillering phase at optimum zinc content in the substrate. Conversely, at higher zinc levels, MeJA seed treatment reduces its inhibitory effect on plant growth and transpiration rate.

1 Introduction

Zinc is an essential microelement for plants. However, its high concentration causes toxic effects. Increases in zinc levels in soil can occur naturally as a result of rock weathering, forest fires and volcanic eruptions, as well as from elevated anthropogenic load. Industrial activity is therefore one of the main sources of zinc. In addition, the regular use of mineral fertilizers containing zinc, and the uncontrolled use of herbicides and pesticides significantly increases the area of contaminated soil [1]. Zinc excess negatively affects plants, slowing down their growth and development, reducing productivity [2]. Therefore, research aimed at finding ways to enhance plant tolerance to zinc is of particular interest.

Phytohormone plant treatment is known to increase plant resistance to biotic and abiotic environmental factors. Various methods of such treatment are currently used, but the most common are seed priming, growing plants on solutions containing phytohormones, and foliar treatment by spraying leaves [3-5]. Seed treatment is the most cost-effective, efficient and widely applicable method [3].

Methyl jasmonate (MeJA), a volatile derivative of jasmonic acid, is also involved in regulating plant growth and increasing plant stress resistance [6]. In specific, MeJA seed treatmentwas found to increase productivity, photosynthetic rate and water use efficiency

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[7-9]. For example, exogenous MeJA induced increased resistance to osmotic stress in rice [7], to salinity in celandines and sorghum [8, 9], and to pathogens in tomato [10]. Comparatively less is known about the MeJA effect on heavy metal plant resistance. Thus, there is information that growing wheat plants on MeJA solution increased their resistance to cadmium [5].

In this regard, the aim of the study was to investigate the effect of MeJA seed treatment on wheat integral physiological parameters (growth, photosynthesis, transpiration) of plant resistance to zinc excess.

2 Materials and methods

The studies were carried out on 3 and 7-day-old seedlings of spring wheat (*Triticum aestivum* L.) of the Zlata variety, as well as plants in tillering phase. Seeds were provided by FGBNU Federal Research Centre "Nemchinovka". The first stage of the study was to determine the optimum for seed treatment concentration of MeJA. For this purpose, seed germination energy and germination capacity were evaluated in specific experiments. Wheat seeds were soaked in different MeJA concentrations (0.001-100 μM) for 24 hours. Seed germination energy was determined on the 3rd day and germination capacity on the 7th day. For further experiments the 10 μM MeJA concentration was chosen.

The effect of 10 μm MeJA seed treatment on plant physiological parameters was investigated at the next stage of the study. Seeds were germinated in Petri dishes with distilled water for 3 days under laboratory conditions. Simultaneously, some seeds were germinated on MeJA solution for 24 h (experiment). The other part of the seeds (control) was germinated on distilled water. Both variants were then transferred to vegetative conditions for further cultivation. Plants (12 per container) were planted in containers with sand (5 litres). Zinc ($ZnSO_4 \cdot 7H_2O$) was preliminarily applied to the sand at the optimum (5 mg/kg) or excessive (46 mg/kg –twice the MAC) concentration for agricultural substrates. Half of the Hoagland-Arnon nutrient solution without zinc salt was used for watering. Thus, the study included 4 experimental variants: Zn 5 (control) - seeds without MeJA treatment, plants grown at optimum zinc concentration; Zn 46 - seeds without MeJA treatment, plants grown at elevated zinc concentration; Zn 5 + MeJA - seeds were treated with MeJA, plants grown at optimum zinc concentration; Zn 46 + MeJA - seeds were treated with MeJA, plants grown at elevated zinc concentration.

In the tillering phase, growth (shoot height and 3rd leaf length), photosynthesis (chlorophyll content, stomatal conductance, photosynthetic rate) and water metabolism (transpiration rate) were assessed. The measurements were carried out using non-destructive methods. The following portable instruments were used: N-tester SPAD-502 Plus, "Konica Minolta", Japan (chlorophyll content measurement), HSM-1000 portable CO2 gas exchange and water vapor system ("Walz", Germany) (stomatal conductance, photosynthetic and transpiration rate analyses).

In each experimental variant, there were 10-36 plants per each biological replicate (3 replicates in total), depending on the parameter studied. Tables and figures represent mean values and their standard errors. Differences were considered significant at $P < 0.05$.

3 Results and discussion

At the first stage of the study, it was found that 10 μM MeJA concentration increased both germination energy and germination capacity (Table 1). Other MeJA concentrations did not affect the parameters studied. Based on the data obtained, further studies were conducted using this concentration.

Table 1. Germination energy and germination capacity of wheat seeds treated with different MeJA concentrations

MeJA concentration, μM	Germination energy, % of control	Germination capacity, % of control
0 (control)	100	100
0.001	100±2.7	100± 1.49
0.01	100±3.6	100± 1.03
0.1	100±2.9	100± 0.63
1	100±2.8	100± 1.19
10	**117 ± 1.6***	**119 ± 1.49***
100	100 ± 2.7	100 ± 1.03

Note: * – differences from control are statistically significant at P< 0,05.

The study of the increased zinc content effect showed its strong negative influence on the growth processes of wheat plants in the tillering phase. Thus, the shoot height and the third leaf length of the Zn 46 variant decreased by 17% and 13%, respectively, compared to the Zn 5 variant plants (Table 2). Growth inhibition in this case may be a consequence of the metal's negative effect on cell division and elongation, as well as a redistribution of energy resources and their allocation to energy-demanding processes associated with enhanced plant resistance [11].

It is known that one of the MeJA effects on plants is growth inhibition [12]. This is associated with the ability of MeJA to suppress mitosis [13]. In addition, the regulation of growth processes under MeJA influence may occur through its interaction with other phytohormones, such as abscisic acid [6,15]. In our experiments, under optimal mineral nutrition conditions MeJA seed treatment (Zn 5 + MeJA) resulted in a slight decrease in shoot height compared to the control (Zn 5). However, no similar effect was observed at higher metal content (Zn 46 + MeJA) (Table 2). It is also important to note that in the absence of MeJA treatment, increased zinc concentration (Zn 46) resulted in a decrease in leaf size (by 14% relative to the control), whereas after MeJA seed treatment (Zn 46 + MeJA), the length of the 3rd leaf tended to increase and was maintained at the level of Zn 5 + MeJA. The observed beneficial MeJA effect may be partly explained by the increased expression of genes encoding the glutathione and phytochelatin synthesis, which bind toxic ions, leading to their detoxification [5].

Table 2. Effect of MeJA seed treatment on growth parameters of wheat plants at tillering stage with optimal and increased zinc content

Parameter	Variant			
	Zn5	Zn46	Zn5+MeJA	Zn46+MeJA
Shoot height, cm	32.71 ± 0.68 a	27.28 ± 0.62 c	30.38 ± 0.75 b	28.22 ± 6.47 c
The 3rd leaf length, cm	16.81 ± 0.49 a	14.40 ± 0.49 c	15.51 ± 0.40 ab	15.16 ± 0.51 bc

Note: Here and in Table 3, different letters indicate statistically significant differences between mean values at $P < 0.05$

The study also showed that the chlorophyll content in the plants of the experimental variant (Zn 46) decreased by 11% compared to the control (Zn 5). MeJA seed treatment had no effect on pigment content at either optimum (Zn 5 + MeJA) or elevated (Zn 46 + MeJA) zinc levels (Table 3). Moreover, increased zinc content in the substrate had a negative effect on stomatal conductance. However, even though preliminary MeJA seed treatment resulted in a 13% increase (compared to Zn 5) in stomatal conductance rate in plants of the Zn 5 + MeJA variant, no similar effect was recorded under stress conditions (Zn 46 + MeJA) (Table 3). MeJA is involved in the stomatal apparatus regulation [17]. For example, MeJA has been shown to increase the stomatal conductance of cereal leaves [15, 16], both under optimal growth conditions and under drought or high temperature stress.

Moreover, little or no change in photosynthetic rate was observed regardless of zinc concentration in substrate, as well as MeJA treatment or its absence (Table 3). Since photosynthesis is sensitive to environmental condition changes, the maintenance of its intensity at control levels under increased zinc content may indicate plant adaptation to this stress effect.

Table 3. Effect of MeJA seed treatment on photosynthetic and water metabolism parameters of wheat at tillering stage with optimal and increased zinc content in the substrate

Parameter	Variant			
	Zn 5	Zn 46	Zn 5 + MeJA	Zn46 + MeJA
Chlorophyll content, SPAD units	39.06 ± 0.56 a	35.50 ± 0.53 b	39.34 ± 0.66 a	36.03 ± 0.54 b
Stomatal conductance, mmol m^{-2} s^{-1}	48.20 ± 1.61 b	38.86 ± 6.81 b	54.80 ± 2.33 a	44.38 ± 3.25 b
Photosynthetic rate, μM m^{-2} s^{-1}	7.77 ± 0.21 a	6.94 ± 0.42 a	7.85 ± 0.20 a	6.98 ±0.18 a
Transpiration rate, mmol m^{-2} s^{-1}	0.51 ± 0.02 ab	0.36 ± 0.03 bc	0.57 ± 0.03 a	0.46 ± 0.03 b

Plants showed a slowdown in transpiration rate (30% compared to control) as the zinc content of the substrate (Zn 46) increased. MeJA seed treatment (Zn 46 + MeJA) contributed to a slight reduction in the zinc inhibitory effect on transpiration. Notably, the intensity of this process was only 10% lower than in the control variant (Table 3). Reduced transpiration rate under zinc excess may be associated with impaired water metabolism due to the root system damage [18,19]. MeJA treatment may contribute to the binding of metal ions [20, 21] through the accumulation of group II LEA proteins (or dehydrins), which have been found, for example, in wheat under drought conditions [22].

4 Conclusion

Thus, MeJA seed treatment at a concentration of 10 μM had a prolonged effect on some physiological parameters in wheat plants of Zlata variety grown under increased zinc content conditions. Specifically, in plants with MeJA seed treatment, the growth of the third leaf and the transpiration rate were inhibited to a lesser extent (compared to untreated plants) under stress conditions. Based on the analysis of data from other authors, we can conclude that the observed changes may occurthrough MeJA interaction with other phytohormones, as well as its effect on the activity of some stress proteins and low molecular weight compounds capable of binding zinc ions.

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References

1. H. Kaur, N. Gard, Planta **253**, 129 (2021). https://doi.org/10.1007/s00425-021-03642-z
2. I. C. Vasilachi, V. Stoleru, M. Gavrilescu, Agriculture **13**, 1983 (2023). https://doi.org/10.3390/agriculture13101983
3. M. Rhaman, Sh. Imran, F. Rauf, M. Khatun, C. C. Baskin, Y. Murata, M. Hasanuzzaman, Plants **10**, 37 (2021). https://doi.org/10.3390/plants10010037
4. H. Manzoor, Mehwish, Sh. Bukhat, S. Rasul, M. I. A. Rehmani, S. Noreen, A. Habib-ur-Rehman, Z. U. Zafar, M. Skalicky, W. Soufan, M. Brestic, M. Habib-ur-Rahman, Ch. C. Ogbaga, A. EL Sabagh, Front. Plant Sci., **13**, 860664 (2022). https://doi.org/10.3389/fpls.2022.860664
5. N. S. Repkina, S. A. Murzina, V. V. Voronin, N. M. Kaznina, Biomolecules, **13**, 582 (2023). https://doi.org/10.3390/biom13040582
6. C. Wasternack, B. Hause, Ann. Bot. **111**, 1021–1058 (2013). https://doi.org/10.1093/aob/mct067
7. M. S. Sheteiwy, D. Gong, Y. Gao, R. Pan, J. Hu, Y. Guan, Environ. Exp. Bot. **153**, 236–248 (2018). https://doi.org/10.1016/j.envexpbot.2018.06.001
8. I. Doycheva, BIORISK**17**, 227–240 (2022). https://doi.org/10.3897/biorisk.17.77465
9. T. Mulaudzi, G. Sias, M. Nkuna, N. Ndou, K. Hendricks, V. Ikebudu, A. J. Koo, R. F. Ajayi, E. Iwuoha, Int. J. Mol. Sci. **24**, 10368 (2023). https://doi.org/10.3390/ijms241210368
10. P. Król, R. Igielski, S. Pollmann, E. Kepczynska, J. Plant Physiol. **179**, 122–132 (2015). http://dx.doi.org/10.1016/j.jplph.2015.01.018
11. L. Bücker-Neto, A. L. S. Paiva, R. D. Machado, R. A. Arenhart, M. Margis-Pinheiro, Genet. Mol. Biol. **40** (1), 373–386 (2017). http://dx.doi.org/10.1590/1678-4685-GMB-2016-0087
12. X. Han, M. Kui, K. He, M. Yang, J. Du, Y. Jiang, Y. Hu, J. Exp. Bot. **74** (4), 1176–1185 (2023). https://doi.org/10.1093/jxb/erac441
13. S. Noir, M. Bömer, N. Takahashi, T. Ishida, T. L. Tsui, V. Balbi, H. Shanahan, K. Sugimoto, A. Devoto, Plant Physiol. **161**, 1930–1951 (2013). https://doi.org/10.1104/pp.113.214908
14. G. Jang, Y. Yoon, Y. D. Choi, Int. J. Mol. Sci. **21**, 305 (2020). https://doi.org/10.3390/ijms21010305 et al., 2020
15. H. Walia, C. Wilson, P. Condamine, X. Liu, A. M. Ismail, T. J. Close, Plant, Cell Environ. **30**, 410–421 (2007). https://doi.org/10.1111/j.1365-3040.2006.01628.x
16. Z. Sehar, M. Fatma, Sh. Khan, I. R. Mir, Gh. Abdi, N. A. Khan, Scientifc Reports **13**, 7468 (2023). https://doi.org/10.1038/s41598-023-34682-y
17. Yu. E. Kulupaev, T. O. Yastreb, Appl. Biochem. Microbiol. **57** (1), 119–135 (2021). https://doi.org/10.31857/S0555109921010281 (In Russ.)
18. A. F. Titov, N. M. Kaznina, V. V. Talanova, *Heavy metals and plants*, (Karel. Nauch. Tsentr Ross. Akad. Nauk, Petrozavodsk, 2014).

19. R. Rucińska-Sobkowiak, Acta Physiol. Plant. **38**, 257 (2016). https://doi.org/10.1007/s11738-016-2277-5
20. M. Hanin, F. Brini, C. Ebel, Y. Toda, S. Takeda, K. Masmoudi, Plant Signaling Behav. **6**, 1503 (2011). https://doi.org/10.4161/psb.6.10.17088
21. M. I. Azarkovich, Russ. J. Plant Physiol., **67** (2), 221–230 (2020). https://doi.org/10.1134/S1021443720020028
22. Ch. Allagulova, A. Avalbaev, K. Fedorova, F. Shakirova, Plant Physiol. Biochem. **155**, 676–682 (2020). https://doi.org/10.1016/j.plaphy.2020.07.012