

The effect of Seed Priming with Hydrogen Sulfide on Germination and Biochemical Indicators of Drought Stress in Sunflower Seedlings

Učinak primiranja sjemena sumporovodikom na klijanje i biokemijske pokazatelje sušnog stresa kod klijanaca suncokreta

Lisjak, M., Ocvirk, D., Špoljarević, M., Teklić, T., Liović, I., Špoljarić Marković, S., Volenik, M., Mijić, A.

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Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

THE EFFECT OF SEED PRIMING WITH HYDROGEN SULFIDE ON GERMINATION AND BIOCHEMICAL INDICATORS OF DROUGHT STRESS IN SUNFLOWER SEEDLINGS

Lisjak, M.⁽¹⁾, Ocvirk, D.⁽²⁾, Špoljarević, M.⁽¹⁾, Teklić, T.⁽¹⁾, Liović, I.⁽³⁾, Špoljarić Marković, S.⁽²⁾, Volenik, M.⁽²⁾, Mijić, A.⁽³⁾

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SUMMARY

*As globally the fourth largest oleaginous plant, sunflower (*Helianthus annuus* L.) is particularly vulnerable to water shortages during the germination phase. Various seed-priming techniques and agents have been proven to enhance germination and promote a uniform seedling growth, especially during the abiotic stress conditions. Since hydrogen sulfide (H_2S) has been documented to be involved in plant metabolism, this study investigates its effects on the sunflower seeds' vigor when germinating in the drought-stress conditions. The research was conducted under the controlled conditions using the Apolon hybrid seeds, developed by the Agricultural Institute in Osijek. This study's objective was to determine a physiological role of H_2S in seedlings grown from the seeds that had been previously primed in sodium hydrosulfide (NaHS) solutions, having germinated under the drought-stress conditions. The results imply that the sunflower seedlings are most sensitive to the osmotic stress during the initial germination phase, particularly in the first four days. An increase in the level of osmotic stress while rising the concentrations of PEG 6000 reduced the seed vigor of the tested hybrid. The most notable positive effects of seed priming with a hydrogen-sulfide donor were observed at the low (2.5% PEG) and moderate (5% PEG) levels of osmotic stress. This study's results may provide future guidelines for the use of various H_2S donors as a priming agent, aimed at an enhancement of drought resistance in early growth phases, which is crucial for the production of this strategically important oilseed faced by a significant climate change.*

Keywords: climate change, hydrogen sulfide, seed priming, stress response, sunflower, water shortage

INTRODUCTION

Water plays a crucial role throughout all phases of plant growth and development, particularly during germination, sprouting, and flowering. Therefore, a lack of water, or a drought stress, can significantly impact plant production (Balestrini et al., 2018; Paul and Roychoudhury, 2020). According to Bukhari et al. (2019), drought can reduce crop quality and yield by more than 50 %. Achieving high germination rates and optimal plant density is essential for stable and high yields. To mitigate the effects of unfavorable weather conditions, particularly water shortages, various seed-preparation techniques are applied during the

germination phase and early seedling development. Primed seeds demonstrate a better germination and a more uniform growth under adverse conditions, such as irregular precipitation and drought, especially on saline soils (Kaya et al., 2006). In recent years, seed priming has become a vital strategy for the production of plants resilient to diverse environmental stresses

(1) Prof. Dr. Miroslav Lisjak (mlisjak@fazos.hr), Marija Špoljarević, PhD, Prof. Dr. Tihana Teklić — Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (2) Dijana Ocvirk, PhD, Sanja Špoljarić Marković — Croatian Agency for Agriculture and Food, Center for Seed and Seedlings, Usorska 19, Brijest, 31000 Osijek, Croatia, (3) Ivica Liović, PhD, Mirna Volenik, PhD, Anto Mijić, PhD — Agricultural Institute Osijek, Južno predgrađe 17, 31000, Osijek, Croatia.

(Sen and Puthur, 2020; Liu et al., 2022). There are two main biological sources of hydrogen sulfide in nature: a geological-biological activity and an enzymatic production and release from the living cells (Ma et al., 2019; Szabo, 2019). The plants are constantly exposed to the environmental factors' fluctuations, which can cause stress and affect their growth and development. Volcanic activity releases large concentrations of this gas into the atmosphere (Aiuppa et al., 2005), suggesting that the plants in close proximity, exposed to high concentrations of the gas, have to develop adaptability to such ecological conditions. Hydrogen sulfide (H_2S) can also be released from a decomposition of coastal marine sediments (Hansen et al., 1978) or from the anoxic marsh soils (Morse et al., 1987). Hydrogen sulfide released in this manner will affect the aerial parts of local plants and the roots of marsh plants. Therefore, throughout evolution, the plants have been continuously exposed to the effects of this gas, and the phenomenon was expected to produce a certain physiological role. A research on plant responses to the environmental stress on the cellular and molecular levels has intensified in recent years (Mittler et al., 2022; Priya et al., 2023). Understanding how the plants perceive environmental signals and transmit them through cellular mechanisms to activate adaptive responses is fundamental to plant biology. Recent studies indicate that H_2S plays a vital physiological role in plant cells, particularly under biotic and abiotic stress conditions (Hancock et al., 2011). Although H_2S was previously considered phytotoxic, emerging evidence suggests that it functions as a signaling molecule. In 2013, it was identified as a potential seed-priming agent for the first time (Christou et al., 2013). Numerous studies in plant molecular biology have recognized H_2S as a gaseous signal transmitter actively involved in various biological processes, including seed germination, root development, shoot function, photosynthesis, aging, and overall plant growth. The studies demonstrate that H_2S application enhances plant tolerance to abiotic stressors such as drought, temperature extremes, salinity, and high heavy-metal concentrations (Hancock, 2019; Singh et al., 2020). As the pieces of research progress, H_2S role has shifted from that of a toxic compound to that of a crucial signaling molecule (Hancock et al., 2011; Lisjak et al., 2013; Pandey and Gautam, 2020). Using H_2S as a priming agent has proven beneficial for important agronomic species grown in challenging environmental conditions (Corpas and Palma, 2020).

Sunflower (*Helianthus annuus* L.) ranks as the fourth-largest oleaginous plant worldwide, following palm, soy, and rapeseed. According to the Food and Agriculture Organization of the United Nations (2024), 18.495 million tons of sunflower oil were produced in 2021, with production expected to continue to rise. While some researchers regard sunflower as a drought-tolerant crop (Markulj Kulundžić et al., 2016; Mahpara et al., 2019), others consider it sensitive to drought (Sarvari et al., 2016; Hussain et al., 2018). Sunflower is particularly vulnerable to water scarcity during

germination (Amin et al., 2014) and the early stages of growth (Vassilevska-Ivanova et al., 2014). Its resistance to drought depends on various morphological, genetic, and physiological traits. A targeted selection with regard to the specific physiological and morphological characteristics (Baloğlu et al., 2012) allows for a more efficient water use during stressful periods. Alongside selection, the application of environmentally friendly chemical compounds like H_2S , which do not only exhibit fungicidal properties but also enhance tolerance to abiotic stresses such as drought, manifests a great potential for agricultural production.

Given this background, this study's objective was to investigate the influence of the H_2S donor sodium hydrosulfide (NaHS) on the vigor of sunflower seeds germinated under different levels of drought stress. The analysis of growth indicators (germination energy, germination, and seedling weight) assessed whether priming with the H_2S donor could enhance the seed vigor under osmotic stress conditions. Additionally, the examination of free proline content, the intensity of lipid peroxidation, and hydrogen-peroxide levels in hypocotyls provided insights into the protective mechanisms against oxidative stress that involve H_2S as a signaling molecule.

MATERIAL AND METHODS

The research was carried out in laboratory conditions on the seeds of the Apolon sunflower hybrid cultivated by the Osijek Agricultural Institute. The Apolon is an early hybrid with a low and strong stem, tolerant to dominant pathogens, with a genetic potential of grain yield up to 5 t ha^{-1} and an oil content of over 50 % (Krizmanić et al., 2014). For each osmotic-stress variant, 850 sunflower seeds were primed in 500 ml of de H_2O , 0.1, 0.5, 1.0 and 1.5 mM NaHS solution. After 2 h of priming, the seeds were dried at room temperature using a filter paper for the next 24 h. A control was represented by the dry, non-primed seeds.

Per repetition, 50 seeds were germinated on a sterile filter papers soaked with 250 ml of polyethylene glycol 6000 (PEG 6000) solution, with the concentrations of 2.5, 5 and 10%, corresponding to an osmotic potential -0.19, -0.499 and -1.483 MPa. For the control, the same amount of de H_2O was used. In order to maintain moisture, moistened filter papers with seeds were placed and sealed in the nylon bags. Germination was carried out according to the ISTA method (ISTA, 2024), at light at 30 °C for 8 h and in darkness at 20 °C for 16 h. The experiment was carried out in four repetitions.

A germination energy (GE) was determined by counting the germinated seeds four days after the experiment setup, while germination (G) was determined by counting the healthy, normally developed seedlings 10 days after the experiment setup. Both seed-vigor indicators were calculated according to the following formula: $\text{Nr. of developed seedlings} / \text{Nr. of sown seeds} * 100$. They were then expressed in percentage (%).

A total seedlings' weight (SW) was determined subsequent to the establishment of the of G value. The hypocotyls were separated from the seedlings and frozen at -80°C . On the day of analysis, the hypocotyl tissue was homogenized using liquid nitrogen.

Lipid peroxidation (MDA) was determined by measuring the specific lipid-breakdown products that react with thiobarbituric acid (Heath and Packer, 1968). A hypocotyl macerate in the amount of 0.2 g of was weighed, and then 0.1 ml of 0.1% TCA (trichloroacetic acid) was added to the samples. The samples were centrifuged for 5 min. at 6,000 RCF at 4°C . A supernatant was separated, and 0.5 ml of 0.5 % thiobarbituric acid (TBA) in 20% TCA, in a ratio of 1:2, was added to the samples. The samples were incubated in a water bath at 95°C for 30 min., cooled, and centrifuged for 15 min. at 18000 RCF at 4°C . Specific and non-specific absorbance was measured at 532 and 600 nm and the concentration of lipid peroxidation products (mainly malondialdehyde, MDA) was calculated using the extinction coefficient $\varepsilon=155\text{ mM cm}^{-1}$ and expressed as $\text{nM g}^{-1}\text{ FW}$ (fresh weight).

Hydrogen-peroxide (HP) content was determined according to Mukherjee and Choudhuri (1983). One ml of 80% of cold acetone was added to 0.2 g of hypocotyls powder. The samples were homogenized and centrifuged for 3 min. at 1,000 RCF at 4°C . A supernatant was separated, and 0.4 ml of titanium sulfate and 0.5 ml of ammonium hydroxide were added. The samples were centrifuged for 10 min. at 15,000 RCF at 4°C . The precipitate was dissolved by adding 1 ml of 2M sulfuric acid. Subsequent to a ten-minute centrifugation at 15,000 RCF and 4°C , the absorbance at 415 nm was measured. The HP content was calculated using an extinction coefficient of 1.878 mM cm^{-1} , and the final results were expressed as $\text{nM HP g}^{-1}\text{ FW}$.

The content of free proline (PRO) was determined according to Bates et al. (1973). Five ml of 3% sulfosalicylic acid was added to 0.25 g of hypocotyl powder and centrifuged for 15 minutes at 4,000 RCF. One ml of supernatant was mixed with the same volume of acidic ninhydrin reagent and glacial acetic acid. The samples were incubated in a water bath for 1 h at 100°C . After cooling it fast to the room temperature using a cold block, 4 ml of toluene was added. The absorbance of toluene layer containing extracted proline was measured at the 520 nm wavelength. A final concentration of PRO in the samples was calculated while applying a calibration curve, obtained while measuring absorbance in the standards with a known concentration of PRO, expressed as $\mu\text{g g}^{-1}\text{ FW}$.

The experiment was set up as a two-factorial one. Spectrophotometric analyses were performed using the Varian Cary 50 UV-VIS spectrophotometer with the Cary WinUV software. The results obtained from the four independent replicates were analyzed using the Enterprise Guide 7.1 software (SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA), F test, and Fisher's LSD test (least significant difference) were administered.

RESULTS AND DISCUSSION

Due to the unfavorable conditions during growth and development, the plants often produce seeds with a low viability. The old seeds, or a storage of seeds under unfavorable conditions, can also be a reason for a decrease in vigor (Andrić et al., 2004). By applying certain chemical compounds, it is possible to increase the seed viability while intensifying physiological and metabolic processes in the seed. One such technique is priming, in which the seed is partially or fully hydrated with water or low concentrations of various osmotic-active salts and then dried prior to sowing (McDonald, 2000). The seeds can also be primed with other physiologically active compounds such as hormones, simple carbohydrates, amino acids, and active compounds or donors of such compounds that is, by the molecules and ions that are directly involved in the cell-signal transduction pathways, for instance, by hydrogen sulfide and hydrogen peroxide.

Our research investigated the effect of hydrogen sulfide on the growth parameters and stress indicators in the hypocotyls of the Apolon sunflower hybrid, germinated on a different level of osmotic stress. In average for all stress levels, the lowest germination energy was determined in the priming variant of 0.1 mM NaHS, but it did not significantly differ in comparison with the control, priming with water, and with 0.5 mM NaHS (Table 1). During priming, damage to the seed coat can occur due to a difference in osmotic potential between the solution and the seed, resulting in an increased leaching of elements involved in osmoregulation, as well as of amino acids and carbohydrates that also play a significant role in the initial phase of germination (Woodstock, 1988; Ashraf et al., 1998). For the Apolon hybrid, higher concentrations of hydrogen sulfide (1 and 1.5 mM NaHS) positively affected the germination energy. In average for all priming variants, the lowest germination energy was detected at the highest stress level caused by a 10 % PEG solution. Iqbal and Ashraf (2006) found that the sunflower-seed treatments with PEG significantly reduce germination percentage and fresh and dry weight and increase the average germination time by up to 50 %.

Priming variants and applied concentrations of PEG did not significantly affect germination (Table 1). Since germination energy is determined as early as on the fourth day and germination by the tenth day (ISTA, 2015), there was a significant time gap between these two indicators, and after ten days the seedlings were equalized and reached full germination regardless of a stress during initial growth. Therefore, the results pertaining to the germination energy and germination confirm that the seedlings were most sensitive to an osmotic stress in the initial phase of germination, and that occurred when the effect of hydrogen sulfide was most pronounced.

On average, for all the applied priming variants, there was a visible decrease in seedling weight with the increasing stress levels during germination. The differences in the fresh-seedling weight between the seed-priming variants were significant ($p \leq 0.05$) at all stress

levels during germination. A significant negative impact of drought stress during germination on the growth of sunflower seedlings was also confirmed by Fulda et al. (2011). The authors report that, in the sunflower seedlings grown up to the appearance of the first true leaves on the MS medium in the presence of PEG (-0.6 MPa), a significant reduction in the hypocotyl length and the

lower fresh mass of shoots and roots was observed. Dooley et al. (2013) assert that a positive effect of hydrogen sulfide is related to a time required for the seed germination and the mass of root, stem, and leaf tissue. In their research, positive effects of H_2S on the germination time and seedling size and weight were observed in the bean, corn, wheat, and pea seeds.

Table 1. The effect of seed priming, stress during germination, and their interactions on the germination energy (GE, %), germination (G, %), seedling weight (SW, g), levels of lipid peroxidation (MDA, $nM\ g^{-1}\ FW$), hydrogen-peroxide content (HP, $nM\ g^{-1}\ FW$), and the free proline content (PRO, $\mu M\ g^{-1}\ FW$) in the hypocotyls of sunflower seedlings. The data are the averages of four replicates; a two-way ANOVA; F test. The values marked with a different letter (a, b, and c) differ according to the LSD test $p \leq 0.05$.

Tablica 1. Učinak primiranja sjemena, stresa i njihove interakcije na energiju klijanja (GE, %), standardnu klijavost (G, %), masu klijanaca (SW, g), razinu lipidne peroksidacije (MDA, $nM\ g^{-1}\ FW$), sadržaj vodikova peroksida (HP, $nM\ g^{-1}\ FW$) i slobodnoga prolina (HP, $nM\ g^{-1}\ FW$) u hipokotilima klijanaca suncokreta. Podatci su prosjeci četiriju ponavljanja dvofaktorska ANOVA; F test. Vrijednosti označene različitim slovima (a, b i c) razlikuju se prema LSD testu $p \leq 0.05$.

Treatment / Tretman		GE	G	SW	MDA	HP	PRO
Priming / Primiranje	Control / Kontrola	76 ^{ab}	81	0.44 ^{ab}	7.112	0.153	3.731
	H ₂ O	77 ^{ab}	83	0.44 ^{ab}	6.715	0.157	3.312
	0.1 mM NaHS	71 ^b	83	0.41 ^d	7.330	0.153	3.745
	0.5 mM NaHS	76 ^{ab}	82	0.42 ^{cd}	7.337	0.144	3.672
	1.0 mM NaHS	80 ^a	86	0.45 ^a	6.772	0.150	3.443
	1.5 mM NaHS	80 ^a	85	0.43 ^{bc}	6.700	0.159	3.412
	F test	3.59	2.05	6.46	2.28	1.45	1.44
	p	0.0059	0.0818	<0.0001	0.0554	0.2169	0.2189
Stress / Stres	H ₂ O	79 ^a	84	0.55 ^a	4.550 ^d	0.199 ^a	0.914 ^d
	2.5% PEG	81 ^a	83	0.50 ^b	6.090 ^c	0.150 ^b	1.966 ^c
	5% PEG	81 ^a	85	0.39 ^c	6.669 ^b	0.191 ^a	3.688 ^b
	10% PEG	66 ^b	82	0.29 ^d	10.650 ^a	0.161 ^b	7.641 ^a
	F test	26.97	1.25	599.01	242.2	91.33	547.93
	p	<0.0001	0.2980	<0.0001	<0.0001	<0.0001	<0.0001
Priming x Stress / Primiranje x stres	F test	4.26	1.76	7.46	2.29	1.76	1.5
	p	<0.0001	0.0587	<0.0001	0.0103	0.0587	0.1282

In our study, priming with a hydrogen-sulfide donor did not significantly affect the biochemical stress indicators during the germination (MDA, HP and PRO) (Table 1). On the other hand, an osmotic stress during the germination significantly influenced the aforementioned parameters. Proportionally to the increase in stress levels during the germination induced by the PEG, there was a significant increase in lipid peroxidation and free proline content. The highest content of hydrogen peroxide was detected in the seedlings germinated in the presence of water. Li et al. (2012) state that hydrogen peroxide plays a vital role in seed germination, as well as in the growth and development of plants, and in acquiring stress resistance, while hydrogen sulfide is considered

to be a cellular signaling molecule in higher plants. The authors note that hydrogen peroxide increases seed germination percentage and stimulates an increased activity of L-cysteine desulfhydrase, which in turn induces the accumulation of hydrogen sulfide.

A significant impact of the osmotic stress on the germination energy was detected in almost all seed-priming variants, while there was no significant difference in germination between the stress variants in all but in the 1 mM NaHS priming variant (Table 2). Furthermore, in all seed-priming variants, statistically significant differences between the applied stress levels were detected concerning the seedling weight, lipid peroxidation levels, hydrogen peroxide, and the free proline content.

Table 2. The effect of stress during germination on the germination energy (GE, %), germination (G, %), seedling weight (SW, g), lipid peroxidation (MDA, nM g⁻¹ FW), hydrogen-peroxide content (HP, nM g⁻¹ FW), and the free proline content (PRO, μM g⁻¹ FW) in the hypocotyls of sunflower seedlings, based on the seed-priming variants. The data are the averages of four replicates; one-way ANOVA; F test. The values marked with a different letter (a, b, and c) differ according to the LSD test $p \leq 0.05$.

Tablica 2. Učinak stresa u klijanju na energiju klijanja (GE, %), standardnu klijavost (G, %), masu klijanaca (SW, g), razinu lipidne peroksidacije (MDA, nM g⁻¹ FW), sadržaj vodikova peroksida (HP, nM g⁻¹ FW) i slobodnoga prolina (HP, nM g⁻¹ FW) u hipokotilima klijanaca suncokreta prema varijantama primiranja. Podatci su prosjeci četiriju ponavljanja; monofaktorska ANOVA; F test. Vrijednosti označene različitim slovima (a, b i c) razlikuju se prema LSD testu $p \leq 0.05$.

Control / Kontrola	GE	G	SW	MDA	HP	PRO
H ₂ O	84 ^a	86	0.56 ^a	4.253 ^d	0.100 ^c	0.748 ^c
2.5% PEG	77 ^a	78	0.54 ^a	5.681 ^c	0.147 ^b	1.449 ^c
5% PEG	80 ^a	82	0.36 ^b	7.326 ^b	0.190 ^a	4.400 ^b
10% PEG	63 ^b	81	0.27 ^c	11.192 ^a	0.176 ^a	8.328 ^a
F test	13.53	1.76	148	49.32	28.09	76.67
p	0.0004	0.2086	<0.0001	<0.0001	<0.0001	<0.0001
H ₂ O						
H ₂ O	84 ^a	86	0.53 ^a	5.378 ^c	0.125 ^c	0.949 ^d
2.5% PEG	81 ^a	84	0.51 ^a	5.016 ^c	0.155 ^b	1.759 ^c
5% PEG	77 ^a	82	0.42 ^b	6.692 ^b	0.187 ^a	3.275 ^b
10% PEG	66 ^b	80	0.30 ^c	9.774 ^a	0.161 ^{ab}	7.265 ^a
F test	10.59	0.89	53.12	32.11	7.47	164.25
p	0.0011	0.4764	<0.0001	<0.0001	0.0044	<0.0001
0.1 mM NaHS						
H ₂ O	71 ^b	80	0.50 ^a	4.396 ^c	0.097 ^c	0.863 ^c
2.5% PEG	87 ^a	87	0.47 ^b	6.233 ^b	0.159 ^b	2.620 ^b
5% PEG	79 ^{ab}	85	0.39 ^c	7.278 ^b	0.194 ^a	3.446 ^b
10% PEG	50 ^c	83	0.27 ^d	11.415 ^a	0.161 ^b	8.052 ^a
F test	26.78	2.28	109.83	34.96	57.29	89.88
p	<0.0001	0.1319	<0.0001	<0.0001	<0.0001	<0.0001
0.5 mM NaHS						
H ₂ O	67	83	0.53 ^a	4.845 ^c	0.099 ^c	0.877 ^d
2.5% PEG	77	77	0.41 ^b	7.013 ^b	0.157 ^b	2.353 ^c
5% PEG	83	85	0.40 ^b	6.240 ^{bc}	0.189 ^a	3.826 ^b
10% PEG	78	83	0.31 ^c	11.252 ^a	0.131 ^b	7.630 ^a
F test	1.84	0.88	98.41	32.57	13.68	38.2
p	0.1929	0.4783	<0.0001	<0.0001	0.0004	<0.0001
1 mM NaHS						
H ₂ O	86 ^a	90 ^a	0.58 ^a	4.335 ^c	0.111 ^c	1.023 ^d
2.5% PEG	82 ^a	86 ^{ab}	0.54 ^b	6.070 ^b	0.138 ^{bc}	1.725 ^c
5% PEG	85 ^a	91 ^a	0.41 ^c	6.212 ^b	0.189 ^a	3.615 ^b
10% PEG	67 ^b	79 ^b	0.26 ^d	10.472 ^a	0.163 ^{ab}	7.409 ^a
F test	7.44	4.88	190.55	121.6	13.32	325.44
p	0.0045	0.0192	<0.0001	<0.0001	0.0004	<0.0001
1.5 mM NaHS						
H ₂ O	79	82	0.56 ^a	4.092 ^c	0.125 ^b	1.026 ^d
2.5% PEG	85	87	0.46 ^b	6.528 ^b	0.142 ^b	1.892 ^c
5% PEG	83	85	0.39 ^c	6.264 ^b	0.197 ^a	3.565 ^b
10% PEG	74	88	0.30 ^d	9.796 ^a	0.171 ^a	7.164 ^a
F test	1.97	0.81	129.41	41.35	11.88	352.5
p	0.1728	0.5146	<0.0001	<0.0001	0.0007	<0.0001

Vassilevska-Ivanova et al. (2014) concluded that an osmotic stress induced by a direct action of PEG affects a large number of physiological and biochemical properties of sunflower seedlings, such as the seed germination, the length of shoots and roots, proportion of fresh and dry matter, water content, and the accumulation of proline, malondialdehyde, and hydrogen peroxide. Zhang et al. (1996) investigated the effect of free radical scavengers on the antioxidant response in sunflower seedlings under a PEG-induced stress and concluded that the sunflower seedlings were sensitive to the osmotic stress, which resulted in the increased levels of lipid peroxidation. The application of ascorbic acid resulted in a reduction of lipid peroxidation levels, and the authors concluded that the external application of free radical scavengers can reduce damage to cell membranes under stress conditions.

In our research, an increased level of lipid peroxidation, observed at the higher levels of stress during germination (5 and 10 % of PEG), confirmed an oxidative stress in sunflower seedlings (Tables 1 and 2). Under the stress conditions during germination, there was also an increased accumulation of hydrogen peroxide in seedlings, resulting in the lower germination rates and a reduced vigor. Additionally, an increase in the drought-stress levels stimulated the accumulation of free proline as an important mechanism of osmotic conditioning in sunflower seedlings under a PEG-induced osmotic stress. The accumulation of proline in sunflower seedlings increased proportionally with the increasing levels of stress during germination, while priming did not significantly affect the accumulation of this osmolyte for all stress variants. At the highest level of stress during germination, 5% and 10% of PEG, the priming variants did not significantly affect proline accumulation (Table 3).

The role of proline in stress resistance remains controversial, as some authors have reported the high levels of proline in the sensitive cultivars exposed to stress conditions (Premachandra et al., 1995; Sundaresan et al., 1995), while Jacobs et al. (2003) claimed the opposite. Lazcano-Ferrat and Lovatt (1999) state that proline serves as an indicator of water status in plants but not as a measure of tolerance level, while Delauney and Verma (1993) explain that the absence of a positive correlation between proline accumulation and an osmotic-stress tolerance in some plant species does not exclude the role of proline in plant adaptation. It is more likely that other morphological or physiological mechanisms are involved in osmotic regulation. A similar response of sunflower to drought in the seedling stage was noted by Khalil et al. (2016), who reported that the sunflower hybrids with greater osmotic-adjustment capability through proline accumulation had a less stress-induced damage, resulting in the greater shoot and root lengths and a higher shoot mass. The authors conclude that a negative correlation with the morphological properties indicates that a proline content may not be associated with an increased sunflower growth; however, it can enhance survival under stress conditions through an osmotic adjustment and play a significant role in an easier and quicker recovery of the plant subsequent the stress cessation.

A large number of previous studies confirm that a prior initiation of moderate stress in plants is an effective preparation resulting in an increased resistance to abiotic stress through the modification of various metabolic pathways involved in the response to stress conditions. With the same goal, various seed-priming methods, or a direct treatment of plants with moderate chemical agents, are targeted to increase tolerance to stress conditions (Savvides et al., 2016).

Table 3. The effect of seed priming on the germination energy (GE, %), germination (G, %), seedling weight (SW, g), lipid peroxidation (MDA, nM g⁻¹ FW), hydrogen-peroxide content (HP, nM g⁻¹ FW), and proline (PRO, μM g⁻¹ FW) in the hypocotyls of sunflower seedlings, based on the stress variants during germination. The data are averages of four replicates: a two-way ANOVA. F test. The values marked by a different letter (a, b, and c) differ according to the LSD test $p \leq 0.05$.

Tablica 3. Učinak primiranja sjemena na energiju klijanja (GE, %), standardnu klijavost (G, %), masu klijanaca (SW, g), razinu lipidne peroksidacije (MDA, nM g⁻¹ FW), sadržaj vodikova peroksida (HP, nM g⁻¹ FW) i slobodnoga prolina (HP, nM g⁻¹ FW) u hipokotilima klijanaca suncokreta prema varijantama stresa tijekom klijanja. Podatci su prosjeci četiriju ponavljanja: monofaktorska ANOVA; F test. Vrijednosti označene različitim slovima (a, b i c) razlikuju se prema LSD testu $p \leq 0.05$.

H ₂ O	GE	G	SW	MDA	HP	PRO
Control / Kontrola	84 ^a	86 ^{ab}	0.57 ^a	4.253 ^b	0.100	0.748 ^c
H ₂ O	84 ^a	86 ^{ab}	0.53 ^{bc}	5.378 ^a	0.125	0.948 ^{ab}
0.1 mM NaHS	71 ^{bc}	80 ^b	0.50 ^c	4.396 ^b	0.047	0.863 ^{bc}
0.5 mM NaHS	67 ^c	83 ^b	0.53 ^b	4.845 ^{ab}	0.099	0.877 ^{abc}
1 mM NaHS	86 ^a	90 ^a	0.58 ^a	4.335 ^b	0.111	1.022 ^{ab}
1.5 mM NaHS	79 ^{ab}	82 ^b	0.56 ^a	4.092 ^b	0.125	1.026 ^a
F test	5.42	2.99	14.18	3.39	1.45	3.82
p	0.0033	0.0390	<0.0001	0.0247	0.2560	0.0156
2.5% PEG						
Control	77	78	0.54 ^a	5.681 ^{bc}	0.147	1.449 ^c
H ₂ O	81	84	0.51 ^b	5.016 ^c	0.155	1.759 ^{bc}
0.1 mM NaHS	87	87	0.47 ^c	6.233 ^{abc}	0.159	2.620 ^a
0.5 mM NaHS	77	77	0.43 ^d	7.013 ^a	0.157	2.353 ^a
1 mM NaHS	82	86	0.53 ^a	6.071 ^{abc}	0.138	1.725 ^{bc}
1.5 mM NaHS	85	87	0.46 ^{cd}	6.528 ^{ab}	0.142	1.892 ^b
F test	1.51	2.19	20.82	2.83	1.46	17.29
p	0.2360	0.1002	<0.0001	0.0469	0.2505	<0.0001
5% PEG						
Control / Kontrola	80	82	0.35	7.326	0.192	4.399
H ₂ O	77	82	0.42	6.692	0.187	3.275
0.1 mM NaHS	79	85	0.39	7.278	0.194	3.446
0.5 mM NaHS	83	85	0.40	6.240	0.189	3.826
1 mM NaHS	85	91	0.41	6.211	0.189	3.615
1.5 mM NaHS	83	85	0.39	6.264	0.197	3.565
F test	1.37	1.41	2.42	1.86	0.41	1.67
p	0.2829	0.2682	0.0760	0.1511	0.8384	0.1920
10% PEG						
Control / Kontrola	63 ^b	81	0.27	11.191	0.176	8.328
H ₂ O	65 ^b	80	0.30	9.774	0.161	7.265
0.1 mM NaHS	50 ^c	83	0.27	11.415	0.161	8.051
0.5 mM NaHS	78 ^a	83	0.31	11.252	0.131	7.630
1 mM NaHS	67 ^{ab}	79	0.26	10.471	0.163	7.409
1.5 mM NaHS	74 ^{ab}	88	0.30	9.796	0.171	7.164
F test	6.34	1.29	1.81	1.93	2.51	0.77
p	0.0015	0.3106	0.1623	0.1395	0.0681	0.5839

CONCLUSION

An increase in stress levels through the application of increasing concentrations of PEG 6000 reduces seed vigor. The differences in germination energy and germination of the sunflower hybrid *Apolon* confirm that the seedlings were most sensitive to an osmotic stress during the initial germination phase, particularly in the first four days. During that period, a positive effect of seed priming with sodium hydrosulfide was most pronounced.

The osmotic stress increases the level of lipid peroxidation, as well as the content of hydrogen peroxide and free proline, proportionally to an increase in the stress levels induced by a PEG application. Based on the results obtained, it can be concluded that a seed priming with the NaHS can be used to enhance a germination and seedling survival under the drought-stress conditions. The research findings concerning the effect of hydrogen sulfide on a seed vigor, growth and development, and the physiological stress indicators in the sunflower seedlings' hypocotyls may provide future guidelines for breeding processes aimed at an increase in drought resistance, which is of great importance for the production of this strategically important oilseed in the times of rapid and pronounced climate change. Since proline accumulation is considered an important mechanism of drought-stress resistance, we can recommend the sunflower *Apolon* as a drought-resistant hybrid, based on its increased accumulation proportional to the increase in drought stress.

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UČINAK PRIMIRANJA SJEMENA SUMPOROVODIKOM NA KLIJANJE I BIOKEMIJSKE POKAZATELJE SUŠNOG STRESA KOD KLIJANACA SUNCOKRETA

SAŽETAK

*Suncokret (*Helianthus annuus* L.), kao četvrta najvažnija uljarica u svijetu, osobito je osjetljiv na nedostatak vode tijekom faze klijanja. Razne tehnike i sredstva primiranja sjemena pokazale su se učinkovitima u poboljšanju klijanja i poticanju ujednačenoga rasta klijanaca naročito u uvjetima abiotškoga stresa. Budući da je dokazano da sumporovodik (H₂S) sudjeluje u biljnome metabolizmu, ovo istraživanje ispituje njegov učinak na vijabilnost sjemena suncokreta naklijavanoga u uvjetima sušnoga stresa. Istraživanje je provedeno u kontroliranim uvjetima korištenjem sjemena hibrida Apolon, proizvedenoga u Poljoprivrednome institutu Osijek. Cilj studije bio je utvrditi fiziološku ulogu H₂S-a u klijancima uzgojenima iz sjemena koje je prethodno bilo tretirano otopinama natrijeva hidrosulfida (NaHS) i naklijavano u uvjetima sušnoga stresa. Rezultati pokazuju da su klijanci suncokreta najosjetljiviji na osmotski stres tijekom početne faze klijanja, osobito tijekom prva četiri dana. Povećanje razine osmotskoga stresa rastućim koncentracijama PEG- 6000 smanjilo je vijabilnost sjemena testiranoga hibrida, dok su najznačajniji pozitivni učinci primiranja sjemena s donatorom sumporovodika, zabilježeni pri niskim (2,5 % PEG-a) i umjerenim (5 % PEG-a) razinama osmotskoga stresa. Rezultati ovoga istraživanja mogu poslužiti kao buduće smjernice za korištenje različitih donatora H₂S-a kao sredstva za primiranje sjemena s ciljem poboljšanja otpornosti na sušu u ranim fazama rasta, što je ključno za proizvodnju ove strateški važne uljarice suočene s izazovima značajnih klimatskih promjena.*

Ključne riječi: klimatske promjene, sumporovodik, primiranje sjemena, odgovor na stres, suncokret, nedostatak vode

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