The influence of hydrogen sulfide on wheatgrass (*Triticum æstivum* L.) de- etiolation

Utjecaj sumporovodika na deetiolaciju pšenične trave *(Triticum æstivum* L.*)*

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THE INFLUENCE OF HYDROGEN SULFIDE ON WHEATGRASS (*Triticum æstivum* L.) DE-ETIOLATION

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SUMMARY

Hydrogen sulfide (H_2S) is involved in many physiological processes and responses to the abiotic types of stress. The aim of the study was to determine the effect of sodium hydrogen sulfide (NaHS) and the time of application on the physiological properties of etiolated wheatgrass plants. Two genotypes of wheatgrass were grown under controlled conditions for five days without light and then with a 12-hour photoperiod, watered for three consecutive days with 100, 200, and 500 mM NaHS solutions. The plants were watered in three variants, 7-9, 10-12, and 13-15 days after sowing, respectively. The highest content of phenols, flavonoids, and hydrogen peroxide was found in wheatgrass plants watered with 100 mM of NaHS solution. The highest proline content and lipid peroxidation levels were found in the plants at 500 mM of NaHS solution. Also, the significant influence of the watering period on the examined physiological parameters was determined. The results show that H_2S significantly affects the de-etiolation process and concentration of physiologically active compounds in wheatgrass plants.

Keywords: NaHS, light stress, antioxidant activity, total phenolics, total flavonoids, DPPH

INTRODUCTION

It is known that wheatgrass, i.e., the young shoots of wheat (*Triticum aestivum* L.) from the family *Poaceae*, is a rich source of vitamins and minerals and has a high antioxidant effect, as well as a high concentration of chlorophyll, flavonoids, and amino acids. Precisely, wheatgrass is used in the prevention and treatment of chronic diseases because of the aforementioned properties (Rana et al., 2011; Singh et al., 2012; Chauhan, 2014; Payal et al., 2015), for it helps in the treatment of diabetes (Thammana et al., 2016) and cancer (Aydos et al., 2011; Tandon et al., 2011; Gore et al., 2017), has an anti-allergic effect (Padalia et al., 2010), it diminishes menopausal symptoms in women (Kumar and Iyer, 2017), helps treat thalassemia (Desai et al., 2008), and demonstrates many other impacts.

The stress stimulates the formation of reactive oxygen species (ROS), resulting in the occurrence of oxidative stress in cells. The plants have antioxidant mechanisms to remove the excessive ROS, thus preventing the cell damage. Over the centuries, H_2S has been exclusively known for its toxicity and environmental hazards (Wang, 2012), but today it emerges as an important signaling molecule (Hancock et al., 2011; Li et al., 2016; Zhang, 2016), participating in the seed germination, plant growth, and development, as well as in the acquisition of stress tolerance, including a cross-adaptation in plants (Li et al., 2016). The protective role

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of hydrogen sulfide in the osmotic (Zhang et al., 2010), drought (Kolupaev et al. 2019a; Batista et al., 2020), heat (Min et al., 2016), and heavy metal stress (Rizwan et al., 2019; Zanganeh et al., 2019; Kaya et al., 2020) has been confirmed. So far, only a few studies have been conducted to examine the effects of hydrogen sulfide in the plants exposed to an excessive photon flux (Joshi et al., 2020; Liu et al., 2019). Fan et al. (2014) concluded that the protective effect of H_2S in plants exposed to the light stress depends on its concentration.

This study aimed to determine the influence of different H_2S concentrations and time of application on the physiological response of etiolated wheatgrass seedlings.

MATERIAL AND METHODS

Plant material

The research was conducted on two varieties of wheat, the French *Renan* and the Italian *Libellula*, from the year 2013-14, provided by the Faculty of Agrobiotechnical Sciences Osijek within the project entitled *Creating Wheat for the Future: The Search for the New Genes from the Existing Sources*, financed by the Croatian Science Foundation (HRZZ). The wheatgrass varieties were chosen based on the level of total antioxidative activity, evaluated in the preliminary research within the project entitled *Genotypic Specificity of Wheatgrass (Triticum æstivum* L.) *High-Nutrient Supplement*. The *Renan* variety belongs to the group with the highest total antioxidant activity, while the *Libellula* belongs to the group with the lowest antioxidant activity.

Growing of wheatgrass

The wheat seeds were washed a few times using the deionized water and sown into the substrate in plastic containers. To induce the etiolation of seedlings, in the first five days the plants were grown in the darkness under the fully controlled conditions in the growth chamber, with a relative humidity amounting to 65% and at a constant air temperature amounting to 20°C. From the sixth day after sowing, a photoperiod was set up at 12 hours. The plants were watered for three consecutive days (7-9, 10-12, and 13-15 days after sowing) with 30 mL of 100, 200, and 500 mM NaHS solutions. The control was watered with the same amount of tap water. On the fifteenth day, the plants were cut off two cm above the ground, the plant material was collected and stored at -80°C for further analysis after grinding in a mortar, and pestled with liquid nitrogen for further analysis. Two hundred seeds per replication were germinated, and the experiment was set up in four replicates.

The analysis of chloroplast pigment content, total phenols, and flavonoids content, ascorbic acid content, free proline content, hydrogen peroxide content, lipid peroxide level and free radical scavenging (DPPH) in wheatgrass

The content of chloroplast pigments (chlorophyll a, chlorophyll b, and carotenoids) was determined spectrophotometrically according to Holm and Wettstein (Holm, 1954; Wettstein, 1957). To detect the total content of phenols and flavonoids, 0.1 g of plant tissue was extracted with 1 mL of 70% ethanol for 48 hours at -20°C. The flavonoids were detected according to Ordonez et al. (2006). The phenols were detected according to Singleton and Rossi (1965). The concentration of blue complex with the Folin-Ciocalteu reagent was measured spectrophotometrically at 765 nm and compared with the absorbance of standard gallic acid (GA) solutions.

Ascorbic acid content was determined spectrophotometrically at 520 nm according to Roe and Kuether (1943), with some modifications. Wheatgrass was pulverized in the liquid nitrogen, and 0.3 g was extracted in the distilled water. By adding 13.3% trichloroacetic acid and 2% dinitrophenylhydrazine-thiourea-copper sulfate reagent in the sample extracts, the ascorbic acid was transferred to a red bis-hydrazone during the incubation time amounting to three hours at 37°C. After incubation, 65% of sulfuric acid was added. The calibration curve was delineated using the ascorbic acid solution as a standard.

A free proline content in 0.5 g of wheatgrass powder was determined according to Bates et al. (1973).

The concentration of hydrogen peroxide in 0.1 g of wheatgrass powder was determined by measuring the amount of titanium peroxide complex, which was deposited when the titanium (IV) oxysulphate sulfuric acid solution and 25% ammonium hydroxide solution were added to the plant extract (Mukherjee and Choudhouri, 1983). The absorbance was measured at 415 nm against a blank sample. The concentration of H_2O_2 was determined using an extinction coefficient amounting to 1.878 mM^{-1} cm^{-1}.

A lipid peroxidation was determined according to Heath and Packer (1968). The concentration of the lipid peroxidation product malondialdehyde (MDA) was calculated by using the molar extinction coefficient of 155 mM^{-1} cm^{-1}.

The wheatgrass' free radical-scavenging activity was measured using the method described by Brand-Williams et al. (1995). The measured wheatgrass' DPPH scavenging properties have been correlated to the amount of ascorbic acid with a known concentration.

Statistical analysis

The obtained data were statistically analyzed using the SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA, 2017) software. The influence of the examined treatments (variety, solution concentration, and the

time of solution application) on the investigated properties was determined by a factorial analysis of variance ($P \leq 0.05$). The differences between the treatments were determined using Tukey's honestly significant difference test (HSD).

RESULTS AND DISCUSSION

In the absence of light, thylakoid membranes do not develop. The plants grown in the dark are elongated and etiolated (Lazarević and Poljak, 2019). Such plants are irresistible and susceptible to various abiotic and biotic stresses. Fan et al. (2014) investigated the effect of hydrogen sulfide on the orchid plants (*Dendrobium officinale*) grown under a high light stress. Watering the plants with 200 μM of NaHS solution was circumstantiated to have exerted a positive effect on the level of maximum photochemical quantum yield of PSII. On the contrary, Liu et al. (2019) confirmed a positive effect of different concentrations of NaHS solutions on the reed wig plants (*Festuca arundinacea* Schreb.) grown in the low-light conditions. In the leaves of plants grown at the low light and treated with the NaHS solutions, a significant increase of chloroplast pigments was found. In our experiment, according to the F test, the content of chlorophyll a and chlorophyll b was under the influence of variety, watering term, NaHS solution and all their interactions (Table 1). The strongest effect on chlorophyll a, total chlorophyll, and carotenoids content was found in the NaHS treatment, compared to the effects of variety and the watering term. Chlorophyll b was under the strongest influence of the variety, which oppositely has not exerted a significant effect on the content of carotenoids (Table 1).

Table 1. The significance of the effect of variety, watering term, NaHS solution, and their interactions on the content of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b) and carotenoids (Car) (mg g^{-1} FW) in the wheatgrass leaves. ANOVA, F test

Tablica 1. Značajnost utjecaja sorte, termina zalijevanja, otopine NaHS i njihovih interakcija na sadržaj klorofila a (Chl a), klorofila b (Chl b), ukupnih klorofila (Chl a+b) i karotenoida (Car) (mg g^{-1} FW) u listu pšenične trave. ANOVA, F test

	Chl a		Chl b		Chl a+b		Car	
	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F
Variety / *Sorta*	8.29	0.0052	39.39	<.0001	13.36	0.0005	1.97	0.1643
Watering term *Termin zalijevanja*	9.46	0.0002	7.02	0.0016	8.74	0.0004	4.78	0.0113
Solution / *Otopina*	24.20	<.0001	15.42	<.0001	22.22	<.0001	12.62	<.0001
Variety*watering term *Sorta*termin zalijevanja*	6.95	0.0017	4.08	0.021	6.23	0.0032	1.50	0.2295
Variety*solution *Sorta*otopina*	5.01	0.0033	4.90	0.0037	5.02	0.0032	2.20	0.0949
Watering term*solution *Termin zalijevanja*otopina*	9.32	<.0001	8.98	<.0001	9.28	<.0001	9.03	<.0001
Variety*watering term*solution *Sorta*termin zalijevanja*otopina*	4.27	0.001	3.75	0.0027	4.17	0.0012	1.70	0.1325

The variety *Libellula*, watered between the seventh and the ninth and between the tenth and the twelwth day, respectively, has had the lowest chlorophyll content at 500 mM of NaHS solution, while between the thirteenth and the fifteenth day there were no significant differences found between the applied NaHS solutions (Table 2). The *Renan* variety, watered with 500 mM of NaHS solution, showed the lowest content of total chlorophyl 1 between the seventh and the ninth day. In general, a 500 mM NaHS solution, applied between the seventh and the nineth day, has significantly decreased the content of chlorophylls, whereas the *Renan* variety was found to be more susceptible, as compared to the *Libellula*. In average for both cultivars, the content of carotenoids in the plants watered in the first watering period was the lowest at 500 mM of NaHS solution, while a significant increase was found in the two later watering periods (Table 2).

Table 2. The Influence of wheatgrass variety (*Libellula*, *Renan*), watering term (seventh to nineth, tenth to twelwth, and thirteenth to fifteenth day after sowing, respectively), and the concentration of NaHS solution (control, 100, 200, and 500 mM NaHS) on the content of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b) and carotenoids (Car) (mg g^{-1} FW) in the wheatgrass leaves. The data are an average of four replicates; Tukey's HSD test

Tablica 2. Utjecaj sorte pšenične trave (Libellula, Renan), termina zalijevanja (7. – 9., 10. – 12., 13. – 15. dan nakon sjetve) i koncentracije otopine NaHS (kontrola, 100, 200 i 500 mM NaHS) na sadržaj klorofila a (Chl a), klorofila b (Chl b), ukupnih klorofila (Chl a+b) i karotenoida (Car) (mg g^{-1} FW) u listu pšenične trave. Podatci su prosjek četiriju ponavljanja; Tukey HSD test

Variety *Sorta*	Watering term *Termin zalijevanja*	Solution / *Otopina*	**Chl a**	**Chl b**	**Chl a+b**	**Car**
Libellula	7^{th}-9^{th}	Control	0.78±0.067	0.24±0.019	1.02±0.085	0.31±0.032
		100 mM NaHS	0.91±0.052	0.28±0.023	1.20±0.073	0.35±0.021
		200 mM NaHS	0.91±0.062	0.27±0.014	1.18±0.077	0.35±0.022
		500 mM NaHS	0.66±0.033	0.21±0.014	0.87±0.047	0.24±0.080
	10^{th}-12^{th}	Control	1.00±0.136	0.31±0.043	1.31±0.178	0.38±0.051
		100 mM NaHS	0.74±0.256	0.24±0.083	0.99±0.338	0.36±0.067
		200 mM NaHS	0.78±0.103	0.25±0.031	1.03±0.134	0.30±0.047
		500 mM NaHS	0.67±0.043	0.23±0.013	0.90±0.055	0.30±0.026
	13^{th}-15^{th}	Control	0.89±0.207	0.26±0.061	1.16±0.267	0.35±0.075
		100 mM NaHS	0.86±0.088	0.26±0.016	1.12±0.103	0.34±0.013
		200 mM NaHS	0.77±0.073	0.24±0.019	1.01±0.091	0.28±0.083
		500 mM NaHS	0.84±0.111	0.28±0.042	1.12±0.154	0.35±0.042
Renan	7^{th}-9^{th}	Control	0.85±0.021	0.25±0.007	1.09±0.027	0.32±0.006
		100 mM NaHS	0.61±0.298	0.18±0.073	0.79±0.371	0.37±0.126
		200 mM NaHS	0.92±0.083	0.25±0.028	1.17±0.110	0.34±0.026
		500 mM NaHS	0.12±0.043	0.05±0.013	0.17±0.056	0.11±0.016
	10^{th}-12^{th}	Control	0.89±0.059	0.26±0.015	1.14±0.073	0.33±0.011
		100 mM NaHS	0.78±0.028	0.23±0.006	1.01±0.033	0.30±0.013
		200 mM NaHS	0.85±0.046	0.24±0.013	1.09±0.059	0.32±0.016
		500 mM NaHS	0.63±0.060	0.20±0.017	0.82±0.078	0.28±0.016
	13^{th}-15^{th}	Control	0.89±0.021	0.23±0.006	1.12±0.026	0.36±0.013
		100 mM NaHS	0.89±0.050	0.24±0.015	1.12±0.064	0.35±0.010
		200 mM NaHS	0.80±0.135	0.22±0.036	1.01±0.172	0.32±0.054
		500 mM NaHS	0.80±0.068	0.22±0.012	1.03±0.079	0.34±0.019
Tukey HSD		Variety*watering term	0.191	0.0516	0.2427	0.0692
		Variety*solution	0.210	0.0580	0.2667	0.0782
		Solution*watering term	0.242	0.0755	0.3146	0.0842
		Variety*watering term*solution	0.304	0.0879	0.3906	0.1268

According to the F test, the total phenols and flavonoids were most significantly influenced by the wheatgrass variety (Table 3). All the interactions have significantly affected the content of flavonoids. In both examined cultivars, watered with 100 mM of an NaHS solution between the tenth and the twelwth day, a significant increase in the content of flavonoids was detected (Table 4). On average for all the NaHS solutions applied, a wheatgrass of the *Renan* variety, watered between the seventh and the nineth day and between the thirteenth and the fifteenth day, respectively, showed a significant increase in the total

flavonoid content. Furthermore, in the *Renan* variety, watered with 100 mM of NaHS between the tenth and the twelwth day, the highest flavonoid content was followed by the lowest level of lipid peroxidation (Table 4). Kolupaev et al. (2019b) stated that the influence of hydrogen sulfide on the flavonoid content is still insufficiently investigated; however, several previous studies confirmed a positive influence of hydrogen sulfide on the content of biological compounds. It is hypothesized that the flavonoids may be among the leading mechanisms concerning a plant's stress resistence under the influence of hydrogen sulfide.

The content of ascorbic acid and free proline was significantly affected by all applied treatments and their interactions (Table 3). The watering term had the most significant effect on the hydrogen peroxide content, while a total antioxidant activity was significantly influenced by the variety.

Table 3. The significance of variety effect, watering day, NaHS solution, and their interactions on the total content of phenols (PH; μg GA 100^{-1} mg^{-1} FW), flavonoids (FL; μg QC 100^{-1} mg^{-1} FW), ascorbic acid (AA; μg 100^{-1} mg^{-1} FW), proline (PRO; μmol g^{-1} FW), hydrogen peroxide (HP nmol g^{-1} FW), lipid peroxidation levels (MDA; nmol MDA g^{-1} FW) and total antioxidant activity (DPPH; mg IC 50%) in the wheatgrass leaves. ANOVA, F test

Tablica 3. Značajnost utjecaja sorte, dana zalijevanja, otopine NaHS i njihovih interakcija na ukupni sadržaj fenola (PH; μg GA 100^{-1} mg^{-1} Sv.T.), flavonoida (FL; μg QC 100^{-1} mg^{-1} Sv.T.), askorbinske kiseline (AA; μg 100^{-1} mg^{-1} Sv.T.), prolina (PRO; μmol g^{-1} Sv.T.), vodikovoga peroksida (HP nmol g^{-1} Sv.T.), razinu lipidne peroksidacije (MDA; nmol MDA g^{-1} Sv.T.) i ukupnu antioksidativnu aktivnost (DPPH; mg IC 50%) u listu pšenične trave. ANOVA, F test

	PH		FL		AA		PRO		HP		MDA		DPPH	
	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F
Variety / *Sorta*	48.8	<.0001	43.69	<.0001	21.61	<.0001	12.94	0.0006	10.34	0.0002	1.1	0.2986	125.72	<.0001
Watering term *Termin zalijevanja*	4.94	0.0097	0.66	0.5175	4.62	0.0013	29.85	<.0001	121.31	<.0001	6.56	0.0024	1.66	0.1974
Solution / *Otopina*	2.95	0.0385	6.69	0.0005	17.05	<.0001	32.28	<.0001	3.09	0.0325	35.49	<.0001	3.11	0.0315
Variety*watering term *Sorta * termin zalijevanja*	2.42	0.0957	9.36	0.0002	5.98	0.004	15.6	<.0001	134.47	<.0001	13.64	<.0001	0.71	0.4929
Variety*solution *Sorta*otopina*	0.03	0.9941	3	0.0362	8.56	<.0001	12.96	<.0001	2.56	0.062	4.62	0.0052	0.93	0.4309
Watering term*solution *Termin zalijevanja* Otopina*	0.67	0.6713	2.57	0.0258	2.73	0.0192	15.05	<.0001	1.85	0.1007	4.99	0.0003	3.45	0.0047
Variety*watering term*solution *Sorta*termin zalijevanja* otopina*	1.41	0.2215	4.34	0.0009	1.31	0.2636	14.49	<.0001	2.53	0.0279	6.66	<.0001	3.66	0.0031

On average, the content of flavonoids, vitamin C, and antioxidant activity for all the watering variants and all NaHS solutions were significantly higher in the *Renan* variety. Zhang et al. (2013) reported the synergistic interactions among the ascorbic acid, ferulic acid, and flavonoids in wheat seedlings, followed by an increase of total antioxidant activity measured by a DPPH and ABTS method. Their research showed that an antioxidant activity depends on the concentration and ratio of the aforementioned compounds, whereas the highest influence was that of the flavonoids.

On average, for both cultivars and the watering terms, the proline content and the lipid peroxidation levels were significantly higher in the plants watered with 500 mM NaHS. Also, on average for all H_2S treatments tested, in the *Renan* wheatgrass, watered between the thirteenth and the fifteenth day, and a higher content of proline was followed by a lower level of lipid peroxidation (Table 4).

Kolupaev et al. (2019a) studied the effect of hydrogen sulfide on the antioxidant status of young winter wheat plants (*Triticum æstivum* L. Doskonala) due to drought stress. A pretreatment with the NaHS prevented the accumulation of hydrogen peroxide and lipid peroxidation caused by a drought stress. The NaHS treatment also resulted in a significant increase of proline, anthocyanins, and flavonoids content.

Table 4. The influence of wheatgrass variety (*Libellula*, *Renan*), watering day (seventh to nineth, tenth to twelwth, and thirteenth to fifteenth day after sowing, respectively) and the concentration of an NaHS solution (control, 100, 200 and 500 mM NaHS) on a total content of phenols (PH; *μ*g GA 100^{-1} mg^{-1} FW), flavonoids (FL; *μ*g QC 100^{-1} mg^{-1} FW), ascorbic acid (AA; *μ*g 100^{-1} mg^{-1} FW), proline (PRO; *μ*mol g^{-1} FW), hydrogen peroxide (HP nmol g^{-1} FW), and lipid peroxidation levels (MDA; nmol MDA g^{-1} FW) and a total antioxidant activity (DPPH; mg IC 50%) in the wheatgrass leaves . The data are an average of four replicates; Tukey HSD test

Tablica 4. Utjecaj sorte pšenične trave (Libellula, Renan), dana zalijevanja (7. – 9., 10. – 12., 13. – 15. dan nakon sjetve) i koncentracije otopine NaHS (kontrola, 100, 200 i 500 mM NaHS) na ukupni sadržaj fenola (PH; μg GA 100^{-1} mg^{-1} Sv.T.), flavonoida (FL; μg QC 100^{-1} mg^{-1} Sv.T.), askorbinske kiseline (AA; μg 100^{-1} mg^{-1} Sv.T.), prolina (PRO; μmol g^{-1} Sv.T.), vodikovoga peroksida (HP nmol g^{-1} Sv.T.), razinu lipidne peroksidacije (MDA; nmol MDA g^{-1} Sv.T.) i ukupnu antioksidativnu aktivnost (DPPH; mg IC 50%) u listu pšenične trave. Podatci su prosjek četiriju ponavljanja; Tukey HSD test

Variety *Sorta*	Watering term *Termin zalijevanja*	Solution *Otopina*	PH	FL	AA	PRO	HP	MDA	DPPH
Libellula	7^{th}-9^{th}	Control	217.78±50.85	104.48±13.19	0.80±0.098	0.57±0.170	9.31±1.10	27.43±1.20	19.15±6.54
		100 mM NaHS	218.73±16.35	98.84±8.65	0.86±0.113	0.69±0.197	8.48±1.22	23.99±3.86	17.85±2.85
		200 mM NaHS	207.48±13.37	92.23±5.50	0.93±0.118	0.56±0.279	7.83±0.43	23.11±3.17	20.70±3.47
		500 mM NaHS	241.77±7.89	101.29±2.46	0.78±0.064	1.04±0.164	7.87±0.61	33.05±2.75	18.81±4.22
	10^{th}-12^{th}	Control	192.83±83.94	128.44±14.50	0.74±0.114	0.27±0.033	11.75±1.38	25.31±0.63	14.65±0.22
		100 mM NaHS	283.21±124.08	165.39±59.59	0.84±0.156	0.56±0.033	7.40±4.21	31.09±12.64	10.83±4.89
		200 mM NaHS	199.38±10.83	91.69±7.03	0.75±0.104	0.45±0.061	7.72±0.98	27.10±1.54	18.08±1.96
		500 mM NaHS	242.28±30.21	104.98±6.49	0.71±0.061	0.98±0.380	9.21±0.81	35.79±4.08	19.50±1.06
	13^{th}-15^{th}	Control	191.60±14.24	100.65±12.34	0.63±0.112	0.27±0.028	7.37±2.21	23.23±2.21	22.12±8,40
		100 mM NaHS	177.41±63.18	112.93±12.93	0.75±0.063	0.34±0.096	8.68±0.74	25.74±4.49	20.23±1.52
		200 mM NaHS	149.50±89.43	101.05±6.85	0.76±0.042	0.40±0.062	8.54±0.37	24.36±0.92	21.27±6.62
		500 mM NaHS	176.96±90.71	127.09±17.44	0.62±0.085	0.60±0.043	8.82±1.68	34.58±6.15	6.38±4.92
Renan	7^{th}-9^{th}	Control	286.22±11.83	167.59±9.09	1.00±0.249	0.33±0.121	16.83±4.19	22.07±2.30	10.97±11.56
		100 mM NaHS	391.21±152.38	232.24±109.42	0.71±0.214	1.45±1.283	21.05±1.20	36.75±20.14	11.12±8.61
		200 mM NaHS	269.12±3.92	164.05±5.06	1.08±0.116	0.21±0.17	15.84±3.62	15.20±1.55	6.40±1.13
		500 mM NaHS	311.16±52.50	114.54±29.33	0.56±0.039	5.58±2.010	18.43±1.98	60.38±2.06	2.04±1.56
	10^{th}-12^{th}	Control	274.59±10.83	77.07±10.17	0.97±0.083	0.38±0.042	5.84±0.53	20.80±2.66	5.48±3.54
		100 mM NaHS	274.24±17.18	161.88±4.29	0.96±0.058	0.35±0.053	6.85±1.92	20.00±1.40	6.61±0.26
		200 mM NaHS	254.44±3.39	149.84±3.64	0.96±0.107	0.32±0.020	4.45±0.43	17.48±2.58	7.96±0.69
		500 mM NaHS	292.11±14.81	128.88±39.72	0.75±0.042	0.81±0.081	5.00±0.58	29.41±2.12	7.56±2.03
	13^{th}-15^{th}	Control	283.82±5.39	169.77±8.10	0.94±0.045	0.33±0.030	6.69±0.51	22.26±2.73	6.09±0.72
		100 mM NaHS	266.57±3.80	150.96±2.78	0.85±0.052	0.34±0.019	5.24±0.84	23.14±6.10	6.41±2.87
		200 mM NaHS	264.72±19.42	147.9±13.02	0.89±0.068	0.45±0.033	5.16±0.96	23.28±2.15	8.61±0.98
		500 mM NaHS	285.56±23.42	141.44±34.06	0.70±0.046	0.60±0.031	5.74±1.43	29.49±1.55	6.12±1.28
Tukey HSD	Variety*watering term *sorta * termin zalijevanja*		58.539	38.207	0.1528	1.0694	2.0917	9.9315	5.5251
	Variety*solution *sorta*otopina*		75.124	46.048	0.1567	1.2517	5.9914	10.311	6.6764
	Watering term*solution *termin zalijevanja* otopina*		118.57	70.404	0.2315	1.4327	6.3693	12.871	12.408
	Variety*watering term*solution *Sorta*termin zalijevanja* otopina*		149.22	78.847	0.2862	1.343	4.8009	15.111	12.09

In general, an increase in the content of free proline was followed by a higher level of lipid peroxidation in all the watering terms in the wheatgrass plants, which were watered with the highest concentration of an NaHS solution. In a water solution, NaHS will dissociate rapidly to generate a very short burst of H_2S, which is a volatile compound and will evaporate quickly. On the other hand, the sodium ions remaining in the substrate after a 500 mM NaHS application could cause an ionic stress, confirmed by the accumulation of an osmoprotective compound, proline.

CONCLUSION

The influence of the sodium hydrogen sulfide and the watering term on all the analyzed physiological properties of etiolated wheatgrass plants were established. The application of 500 mM of an NaHS solution have exerted a negative impact on the tested parameters in both wheatgrass varieties. The negative effect was more pronounced in the first watering term, when the plants were still recovering from the etiolation after being grown in the darkness. According to the obtained results, the *Renan* variety seems to be more tolerant to the etiolation. The lower concentrations of the H_2S applied resulted in a higher accumulation of physiologically active compounds and, consequently, in a more efficient etiolation recovery, confirming a protective H_2S role .

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UTJECAJ SUMPOROVODIKA NA DEETIOLACIJU PŠENIČNE TRAVE (*Triticum æstivum* L.)

SAŽETAK

Sumporovodik (H_2S) je uključen u velik broj fizioloških procesa i reakcija na abiotske tipove stresa. Cilj istraživanja bio je utvrditi utjecaj natrijevog hidrogensulfida (NaHS) i vremena primjene na fiziološka svojstva etioliranih biljaka pšenične trave. Dva su genotipa pšenične trave uzgajana u kontroliranim uvjetima pet dana bez svjetlosti te nakon toga uz dvanaestosatni fotoperiod, zalijevane tri dana zaredom otopinama NaHS koncentracija 100, 200 i 500 mM. Varijante tretmana zalijevanja uz osvjetljenje bile su sedmoga do devetoga, desetoga do dvanaestoga te trinaestoga do petnaestoga dana nakon sjetve. Najveći sadržaj fenola, flavonoida te vodikova peroksida utvrđen je kod biljaka pšenične trave zalijevanih otopinom 100 mM NaHS. Najviši sadržaj prolina i lipidna peroksidacija utvrđeni su kod biljaka pri 500 mM NaHS. Također, utvrđen je i značajan utjecaj perioda zalijevanja na ispitivane fiziološke parametre. Rezultati pokazuju da H_2S značajno utječe na proces deetiolacije kod biljaka pšenične trave i sadržaj fiziološki aktivnih komponenata u pšeničnoj travi.

Ključne riječi: natrij hidrogen sulfid, svjetlosni stres, antioksidativna aktivnost, ukupni fenoli, ukupni flavonoidi, DPPH

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