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ORIGINAL PAPER

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THE WATER TREATED WITH LOW-FREQUENCY LOW-PRESSURE GLOW PLASMA ENHANCES THE PHYTOAVAILABILITY OF SELENIUM AND PROMOTES THE GROWTH OF SELENIUM-TREATED CUCUMBER PLANTS

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ABSTRACT

For its specific physical and physicochemical properties, the water treated with low-frequency low-pressure glow plasma (GPTW) affects the growth of plants and enhances the phytoavalibility of selenium (Se) ions from the nutrient solution. The basic biometric and physiological parameters of cucumber and the uptake of Se ions applied as selenate (Na_2SeO_4) from the nutrient solution prepared using GPTW or distilled water (DW) were compared. In the presence of Se, the fresh weight (f.w.) of shoots of plants growing in water-differentiated nutrient solutions did not differ, whilst their dry weight (d.w.) and leaf area (LA) were higher in plants grown in the GPTW- than in DW-containing medium. The use of GPTW for preparation of the nutrient solution was associated with a substantial improvement of Se ions phytoavailability, compared to the regular growth medium based on DW. Despite the higher Se bioaccumulation in the GPTW- than in DW-based medium, the phytotoxicity of this element was not enhanced. GPTW-induced Se accumulation was remarkable and hence recommended for further study to understand the detailed mechanism GPTW action.

Key words: glow plasma treated water, *Cucumis sativus* L., biofortification, selenate, phytotoxicity

INTRODUCTION

There are 20 mineral elements that are essential for human health, including 7 major elements (Ca, P, K, S, Na, Cl, Mg) and 13 trace elements (Fe, I, Cu Zn, Mn, Co, Cr, Se, Mo, F, Sn, Si, V). These elements cannot be synthesized by the organism and must be supplied from food [Yin et al. 2012]. Micronutrient malnutrition also called "hidden hunger" affects more than one-half of the world's population. Biofortification is the development of micronutrient-enriched edible crops using two strategies: traditional plant breeding (agronomic biofortification) or modern biotechnology (genetic biofortification) [Nestel et

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al. 2006]. Agronomic biofortification is based on application of mineral fertilizers to the rhizosphere or the use of foliar fertilization. It is considered to be a safe and effective way to prevent micronutrient malnutrition in many micronutrient-deficient areas. However, the supply and phytoavailability of mineral elements in the rhizosphere ultimately limit their phytoaccumulation by crops [White and Broadley 2009].

Selenium (Se) is one of the trace elements that are essential for humans and animals. On the other hand, higher plants do not require Se for their proper growth and development although recently it is classified as a beneficial element for plants [Kopsell and Kopsell 2007]. It is estimated that over 15% of the global population are Se-deficient [White and Broadley 2009]. Since Se deficiency in the human diet is a common phenomenon in many countries around the world, crops biofortified with Se are an excellent source of dietary Se than can help to mitigate this problem [Garcia-Banuelos et al. 2011]. Currently, many studies are being conducted for evaluation of the biofortification efficiency of different crop species with Se and the impact of Se on plant metabolism and its nutritional value have been also deeply analysed [Broadley et al. 2006, White and Broadley 2009, Hawrylak-Nowak 2013, Malagoli et al. 2015, Hegedűsová et al. 2017]. The enhanced phytoaccumulation of Se is desired for increased efficiency of the biofortification and different methods are used to achieve this goal. Se biofortification efforts may make use of natural variation between different plant species in their capacity to accumulate Se, and choose crops that tend to contain higher levels of this element, such as *Brassica* or *Allium* species [Malagoli et al. 2015]. Also, the methods of genetic engineering are currently used for the improvement of Se accumulation and tolerance, as well as to provide fundamental knowledge of the uptake, translocation, and metabolism of this element in plants. An interesting new area of research also involves the use of plant-microbe interactions to improve Se biofortification efficiency [Malagoli et al. 2015, White 2015]. Additionally, Se supplementation to plants may also be beneficial to the production and quality of crop plants, enhancing their yield and nutritive value [Haug et al. 2007, Hegedűsová et al. 2017]. Therefore, efforts to increase Se concentration in the plants are urgent for both current and future generations.

Water treated with low-frequency low-pressure glow plasma (GPTW) is colloquially termed nanowater. During this process, water molecules, which form aggregates (clusters) of up to 1000 under standard conditions, are broken down into small, neatly arranged groups, or nanoclusters. Treatment of water or a 0.9% NaCl water solution with low-temperature low-pressure plasma significantly changes their properties such as pH, electrical conductivity, and surface tension. Moreover, GPTW has low viscosity, high diffusivity, and very low density. It dissolves 35–40% more substances than the same volume of normal water, thus it can be a very efficient carrier of mineral nutrients [Mystkowska et al. 2013, Murawski et al. 2015].

The changes in the physicochemical properties of water can potentially alter its biological properties. We hypothesized that these unique features of GPTW would modify the phytoavailability and bioaccumulation of some mineral nutrients, and in consequence affects some physiological processes in plants. To test this hypothesis, for our study we chose Se, due to the large interest in this element uptake and accumulation in the aspect of crop biofotification. Therefore, in this experiments the effect of the water used for preparation of the nutrient solution on Se accumulation and translocation as well as the growth and some physiological parameters of cucumber cultivated by the hydroponic method has been examined. The results of this paper could be useful in improving the efficiency of Se biofortification through the use of GPTW as a carrier of Se.

MATERIALS AND METHODS

Treatment of water with plasma. The water treated with low-temperature low-frequency glow plasma was obtained from Nantes Chemicals (Bolesławiec, Poland). Distilled water was transferred into a chamber reactor and exposed to plasma at 38°C generated at 5×10^{-3} mbar, 600V, 50 mA and frequency of 280 GHz for 90 min. The GPTW

was stored at ambient temperature in the closed Teflon containers [Bialopiotrowicz et al. 2016]. GPTW was produced using equipment patented in 2009 [Oszczęda et al. 2009]. The equipment for GPTW production consisted of a plasma reactor, containing of a truncated-cone vacuum chamber and a liquid container placed inside the chamber. The chamber had the disk-shaped electrodes – the anode at the top and cathode at the bottom, generating a pulsed electric field. The parameters of the electric field were controlled by a probe placed inside the vacuum chamber and connected to the pulse generator.

Plant material, experimental design, and growth conditions. The seeds of cucumber (*Cucumis sativus* L.) cv. Polan F1 germinated in quartz sand at 23–25°C for 7–8 days. Then, uniformly sized plants were transferred to 1 L glass jars filled with a full strength Hoagland's No. 2 nutrient solution (pH = 5.5). The nutrient solution was prepared using distilled water (DW) or distilled water treated with low-frequency low-pressure glow plasma (GPTW). Then, the growth medium was differentiated in regard to the concentration of Se: 0 (control), 10, or 100 μM Se applied as selenate (Na_2SeO_4). We chose concentrations of selenate that are potentially beneficial (10 μM) or toxic (100 μM) for this species [Hawrylak-Nowak et al. 2015]. The experimental design was randomized in a 2 × 3 factorial scheme (DW and GPTW; three concentrations of Se). The experiment included six treatments with six plants per treatment (two plants per each of three jars), and the whole experiment was repeated independently three times under the same conditions. Plants were cultured in a controlled-climate phytotron room under the following conditions: photosynthetic photon flux density at the level of the tops of the plants of 250–270 μmol m^{-2} s^{-1}, 14-h day length, temperature 25/22°C (day/night), and relative humidity of 50–60%. The nutrient solution was aerated for 10 min every two days using an aquarium air pump and was replenished with the appropriate type of water when required. The plants were analyzed after 14 days of growth under differentiated conditions.

Determination of growth parameters. After 14 days of growth, the plants were harvested, divided into roots and shoots, and the fresh weights (f.w.) were determined immediately after harvest. The root systems were rinsed twice in distilled water. Fresh second true leaves were scanned using CI-202 laser area meter (CID Bio-Science, USA) to determine the leaf area (LA). Thereafter, plant materials were dried at 80°C and dry weights (d.w.) of plant organs were determined. The percentage water content in roots and shoots was calculated using the following formula:

$$\% H_2O = (f.w. - d.w.) / f.w. \times 100$$

In total, for determination of growth parameters, we used 18 plants (6 plant form each treatment × 3 independent repetitions).

Determination of photosynthetic pigment concentrations and chlorophyll *a* fluorescence. Chlorophylls *a* and *b* together with carotenoids were determined and calculated according to the method described by Lichtenthaler and Wellburn [1983]. The samples were collected from the second true leaves (2 samples from each treatment × 3 independent repetitions) and pigments were extracted by homogenization with 80% acetone. The absorbance of the resulting solutions was recorded at 646, 663, and 470 nm. The selected chlorophyll *a* fluorescence parameters included the minimal (F_0) and maximal (F_m) level of fluorescence and the maximum quantum yield of PS II (F_v/F_m, where $F_v = F_m - F_0$) [Murchie and Lawson 2013]. These parameters were measured on the same leaves that were used to extraction of photosynthetic pigments using a Handy PEA fluorimeter (Hansated Instruments, Japan). The leaves (4 form each treatment × 3 independent repetitions) were adapted to darkness for 15 min before the measurements by attaching light-exclusion clips.

Analysis of total Se concentrations. For determination the content of Se, we used mixed dry samplers of roots or shoots (1 sample from each treatment × 3 independent repetitions). The dry plant material was subjected to nitric–perchloric acids mineralization (HNO_3–$HClO_4$; 4 : 1). After mineralization, hydride generation atomic absorption spectroscopy (HG-AAS) was used to determine the total Se concentrations in the acid digests. This method is based on Se's conversion to a volatile hydride (SeH_2) by the $NaBH_4$ and aspiration into an atomic absorption spec-

trometer (Perkin Elmer 1100B) fitted with a hydride generation system (Perkin Elmer MHS–10). In brief, after cooling the mineralized samples, digests were quantitatively transferred to the volumetric flasks, 5 M HCl and deionized water were added, and then heated 30 min at 80°C to reduce all Se to four oxidation state. After the reduction, all Se was converted to SeH_2 with 3% $NaBH_4$ in 1% NaOH. The signal was recorded at a wavelength of 196 nm. A certified reference material (WEPAL IPE-157; beech leaf) was used for method validation.

Statistical analyses. The data were statistically analyzed by applying two-way ANOVA with the type of water and Se concentration as experimental factors. Significance of differences was assessed using the Tukey's multiple range test at the confidence level of $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of growth parameters. Statistical analysis of the main effects showed no significant effect of water used for preparation of the nutrient solution on the cucumber root and shoot f.w. and d.w. (tab. 1). On the other hand, the Se application and interaction between the studied factors significantly affected plants biomass. A comparison of the growth of Se-untreated plants revealed that the f.w. and d.w. of the shoots and roots were significantly higher in the DW-based than in the GPTW-based medium. The cause of this phenomenon requires further clarification, but we suppose that it may be associated with better solubility of mineral salts in GPTW than in DW [Murawski et al. 2015], which can modify the bioavailability of essential mineral nutrients from the solution. However, when

Table 1. Growth of cucumber under different concentrations of Se in the nutrient solution prepared using distilled water (DW) or water treated with low-frequency low-pressure glow plasma (GPTW)

Treatments		f.w. (g plant^{-1})		d.w. (g plant^{-1})		Water content (%)		LA of 2nd leaf (cm^2 plant^{-1})	Root length (cm plant^{-1})
Type of water	Se (µM)	shoot	root	shoot	root	shoot	root		
Distilled water (DW)	0	8.24 ±0.92 a	6.36 ±0.79 a	0.757 ±0.89 a	0.182 ±0.02 a	90.8 ±2.4 a	97.14 ±2.8	85.9 ±10.3 ab	26.17 ±6.34
	10	7.13 ±0.65 ab	4.55 ±0.53 c	0.620 ±0.72 b	0.124 ±0.02 b	91.3 ±2.1 a	97.27 ±2.5	81.9 ±6.5 b	25.03 ±4.92
	100	3.55 ±0.41 c	1.15 ±0.09 d	0.301 ±0.26 d	0.029 ±0.01 d	91.5 ±2.6 a	97.48 ±4.2	33.5 ±2.4 d	13.28 ±4.67
Glow plasma treated water (GPTW)	0	6.94 ±0.77 b	4.64 ±0.39 c	0.626 ±0.43 b	0.136 ±0.03 b	91.0 ±2.9 a	97.07 ±1.8	82.8 ±10.9 ab	25.39 ±7.56
	10	7.52 ±0.54 ab	5.51 ±0.64 b	0.726 ±0.66 a	0.179 ±0.04 a	90.4 ±1.8 a	96.75 ±1.6	89.1 ±11.3 a	25.25 ±5.25
	100	3.96 ±0.36 c	1.31 ±0.11 d	0.358 ±0.23 c	0.064 ±0.01 c	84.1 ±3.6 b	95.11 ±2.1	42.8 ±5.1 c	13.13 ±3.98
Main effects									
Water	DW	6.31	4.02	0.559	0.112	91.21 A	97.3	67.0 B	21.49
	GPTW	6.14	3.82	0.570	0.126	88.49 B	96.3	71.6 A	21.25
Se	0	7.59 A	5.50 A	0.692 A	0.159 A	90.90 A	97.1	84.2 A	25.78 A
	10	7.33 A	5.03 A	0.673 A	0.152 A	90.83 A	97.0	85.5 A	25.14 A
	100	3.76 B	1.23 B	0.330 B	0.047 B	87.83 B	96.3	38.2 B	13.21 B
Statistical significance									
Water		NS	NS	NS	NS	*	NS	*	NS
Se		*	*	*	*	*	NS	*	*
Water × Se		*	*	*	*	*	NS	*	NS

The mean values ± SD (n = 18) in each column followed by different letters indicate significant differences between treatments (Tukey's test; $p < 0.05$). * significant difference at $p < 0.05$, NS = not significant

Table 2. Concentration of photosynthetic pigments and selected parameters of chlorophyll a fluorescence in cucumber plants grown under different concentrations of Se in the nutrient solution prepared using distilled water (DW) or water treated with low-frequency low-pressure glow plasma (GPTW)

Treatments		Concentration of photosynthetic pigments (mg g^{-1} f.w.)			Selected parameters of chlorophyll *a* fluorescence		
Type of water	Se (μM)	chlorophyll *a*	chlorophyll *b*	carotenoids	F_0	F_m	F_v/F_m
Distilled water (DW)	0	1.676 ±0.153 b	0.349 ±0.059 b	0.367 ±0.014	347.0 ±24.5	1829 ±65 a	0.810 ±0.031
	10	1.639 ±0.129 b	0.335 ±0.044 b	0.345 ±0.010	353.2 ±46.2	1847 ±42 a	0.810 ±0.024
	100	0.970 ±0.077 c	0.194 ±0.022 c	0.243 ±0.024	337.7 ±67.2	1524 ±33 c	0.778 ±0.034
Glow plasma treated water (GPTW)	0	1.838 ±0.136 a	0.414 ±0.058 a	0.365 ±0.035	367.8 ±19.8	1839 ±51 a	0.809 ±0.026
	10	1.601 ±0.168 b	0.352 ±0.054 b	0.315 ±0.030	343.3 ±43.3	1802 ±37 a	0.809 ±0.042
	100	1.041 ±0.133 c	0.194 ±0.027 c	0.237 ±0.033	356.5 ±55.7	1615 ±25 b	0.780 ±0.024
Main effects							
Water	DW	1.428	0.293	0.318	346.0	1733	0.810
	GPTW	1.493	0.320	0.306	355.9	1752	0.810
Se	0	1.757 A	0.382 A	0.366 A	357.4	1834 A	0.810 A
	10	1.620 A	0.344 A	0.330 A	348.3	1825 A	0.810 A
	100	1.006 B	0.194 B	0.240 B	347.1	1570 B	0.779 B
Statistical significance							
Water		NS	NS	NS	NS	NS	NS
Se		*	*	*	NS	*	*
Water × Se		*	*	NS	NS	*	NS

The mean values (n = 6 for the concentration of photosynthetic pigments, and n = 12 for F_v/F_m value) in each column followed by different letters indicate significant differences between treatments (Tukey's test; $p < 0.05$).* significant difference at $p < 0.05$, NS = not significant

tiated nutrient solutions did not differ statistically. Only the roots of plants exposed to 10 μM grew better in GPTW, as their f.w. and d.w. were by 21% and 44% higher in the GPTW- than in the DW-based medium. In this experiment, we did not find a growth-promoting effect of the lower Se concentration (10 μM) on plants grown in the DW-based nutrient solution, as shown in previous studies, but under somewhat different experimental conditions [Hawrylak-Nowak et al. 2015]. On the other hand, the presence of 10 μM Se in the GPTW-based medium stimulated plant growth, compared to the Se-untreated control grown in GPTW. The concentration of 100 μM Se was markedly toxic for cucumbers, regardless of the type of the water used. Nonetheless, the biomass and LA of the plants treated with Se in general were significantly higher in the growth medium prepared using GPTW than in that using DW (tab. 1).

To determine whether the type of water and Se treatment affect the plant water relations, the percentage of the water content was determined in cucumber organs (tab. 1). The shoot water content was significantly affected by both of the studied factors and interactions between them, but these factors did not influence the root water content. In plants grown in the GPTW-based medium and exposed to 100 μM Se, the percentage of the water content in the above-ground organs was significantly lower than in the other treatments, which may be the cause of the higher shoot d.w. recorded in plants grown in the GPTW- than in the DW-based nutrient solution.

Regardless of the type of the water used for preparation of the medium, the lengths of the root system were reduced by about half in the presence of 100 μM Se, compared to the Se-untreated plants. Additionally, the LA of plants exposed to 100 μM Se was significantly lower. However, the LA of the Se-exposed plants grown in the GPTW-based nutrient solution was greater than that of the plants grown in the DW-based medium (tab. 1).

Concentration of photosynthetic pigments and chlorophyll *a* fluorescence parameters. The level of photosynthetic pigments in the cucumber leaves was determined not by the type of water used for preparation of the nutrient solution, but by the concentration of Se and interactions between these factors (tab. 2). In the control (Se-untreated) plants, the concentrations of chlorophyll *a* and *b* were higher by 10% and 19%, respectively, in the medium based on GPTW than in those growing in the standard DW-containing medium. However, when the cucumber plants were supplied with 10 or 100 μM Se, the level of both chlorophyll forms did not differ significantly between the plants cultivated in the nutrient solutions prepared using the different types of water. Furthermore, we found that addition of 100 μM Se was phytotoxic, as the concentration of chlorophylls decreased by 43–53%, while the level of carotenoids was reduced by 35%. The concentration of 100 μM Se was above the toxicity threshold of selenate for cucumber determined previously, which was 80 μM and at which the decline in the chlorophyll level occurred without significant changes in the carotenoids concentration [Hawrylak-Nowak et al. 2015].

The analysis of chlorophyll *a* fluorescence showed that the type of water used had no effect on the analyzed parameters of fluorescence (tab. 2). However, the 100 μM selenate treatment adversely affected the F_v/F_m value (an indicator of the maximum quantum yield of PSII), which in non-stressed plants is 0.81–0.83 [Murchie and Lawson 2013]. The F_m value (maximal possible value for fluorescence) was also reduced under these conditions; however, this reduction was significantly higher in plants grown in the DW-based than in the GPTW--based medium (tab. 2), which indicates that selenate at the 100 μM concentration was more toxic in the DW-based than in the GPTW-based nutrient solution.

Selenium concentration and translocation. To verify whether the GPTW affects the phytoavailability of Se from the medium, the Se concentration in roots and shoots as well as Se translocation from roots to the aboveground parts were determined. Statistical analysis of the main effects showed a significant impact of water used for preparation of the nutrient solution on the concentration of Se in the individual parts of the plants (tab. 3). Our results imply that plants cultivated in the GPTW-based medium contained more Se in their organs that those grown in the standard nutrient solution. This phenomenon has been particularly pronounced in the roots of plants treated with 100 μM Se. Under these conditions, the content of Se in the roots reached 665 and 1440 mg kg^{-1} d.w., respectively, in the DW- and the GPTW-based nutrient solutions. Also, the aboveground organs accumulated more Se when the plants were grown in the GPTW-based medium, although the Se content was not so substantially elevated as in the case of the roots (tab. 3). Meanwhile, Se phytotoxicity was not intensified at the higher tissue Se concentration noted in the GPTW-based medium, but was even lower, as can be concluded from the results regarding the biomass, LA (tab. 1), and F_m value (tab. 2). The enhanced Se accumulation in the GPTW-based medium, especially in the roots, may result from the higher Se solubility in GPTW than in DW [Murawski et al. 2015] or/and from the presence inside the GPTW clathrates of excited, probably singlet oxygen molecules [Bialopiotrowicz et al. 2016], which can modify the oxidation state and/or balance of Se ions in the medium, thus influencing the availability and translocation of Se within plants. It is known that Se in the form of selenite (Se^{4+}) is rapidly converted to organoselenium compounds that are accumulated in the root system, whereas selenate (Se^{6+}) is delivered immediately to the xylem and assimilated into organoselenium compounds in plastids [White 2015]. The higher concentration of Se in the roots of Se-exposed plants grown in the GPTW-based nutrient solution could suggest that used in our study selenate in the GPTW-containing medium may be reduced to selenite, and therefore Se can be stronger accumulated in the roots. However, this hypothesis requires further detailed research and determination of Se speciation in the nutrient solution and plants. In the Se-untreated plants we also found the small amounts of Se (tab. 3), which may have come from the trace content of this element in the sand used for seed germination and/or form reagents being a source of macro- and micronutrients for Hoagland's nutrient solution.

Table 3. Concentration of total Se and the Se translocation factor (TF) in cucumber plants grown under different concentrations of Se in the nutrient solution prepared using distilled water (DW) or water treated with low-frequency low--pressure glow plasma (GPTW)

Treatments		Selenium concentration (mg kg^{-1} d.w.)		TF (shoot/root Se ratio)
Type of water	Se (μM)	shoot	root	
Distilled water (DW)	0	0.70 ±0.09 e	1.30 ±0.18 e	0.55 ±0.04 d
	10	77.5 ±6.5 d	22.5 ±3.5 d	3.44 ±0.27 a
	100	1050 ±16 b	665 ±43 b	1.58 ±0.11 c
Glow plasma treated water (GPTW)	0	0.20 ±0.04 e	0.70 ±0.11 e	0.30 ±0.08 d
	10	96.5 ±8.4 c	33.0 ±4.5 c	2.92 ±0.48 b
	100	1100 ±23 a	1440 ±57 a	0.76 ±0.14 d
Main effects				
Water	DW	376.1 B	229.6 B	1.86 A
	GPTW	398.9 A	491.2 A	1.33 B
Se	0	0.45 C	1.00 C	0.43 C
	10	87.0 B	27.8 B	3.18 A
	100	1075 A	1053 A	1.17 B
Statistical significance				
Water		*	*	*
Se		*	*	*
Water × Se		*	*	*

The mean values (n = 3) in each column followed by different letters indicate significant differences between treatments (Tukey's test; $p < 0.05$). * significant difference at $p < 0.05$, NS = not significant

CONCLUSIONS

In summary, we suggest that the use of GPTW for preparation of nutrient solutions can be associated with a substantial improvement in Se phytoavailability, and can thus increase the effectiveness of Se biofortification of crop plants. Despite the higher bioaccumulation of Se in plants grown in the GPTW-based medium, the phytotoxicity of Se was not enhanced in this type of water. Moreover, some of the measured biometric parameters of the plants treated with 10 μM Se were positive influenced in the presence of GPTW. The data presented here are novel and represents a promising strategy for enhanced biofortification efficiency of crop plants in order to produce Se enriched foods. However, further studies are needed to elucidate a mechanism by which GPTW increases Se content in plants.

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