Biotechnology Journal International

22(2): 1-11, 2018; Article no.BJI.45202
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Effects of Hydrogen-Rich Water on the Growth and Photosynthetic Characteristics of Cucumber Seedlings

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2018/45202
Editor(s):
(1) Dr. Anil Kumar, Professor, School of Biotechnology, Devi Ahilya University, India.
Reviewers:
(1) Seweta Srivastava, Lovely Professional University, India.
(2) Jose de Jesus Arellano García, Universidad Autonoma del Estado de Morelos, Mexico.
(3) R. K. Mathukia, Junagadh Agricultural University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/27391

Short Research Article

Received 07 September 2018
Accepted 19 November 2018
Published 24 November 2018

ABSTRACT

Aims: The aim of the paper was to study the effect and specific mechanism of hydrogen-rich water (HRW) improving cucumber (*Cucumis sativus*) seedlings growth.
Study Design: 14-day-old cucumber seedlings were treated with different saturation hydrogen-rich water in root for three times every three days. After one day of the last treatment, growth parameters were determined and plants tissues were sampled for test photosynthetic characteristics.
Place and Duration of Study: In 2017, cucumber (*C. sativus* 'JinYan4') were germinated in College of Bioscience and Biotechnology of Shenyang Agricultural University (Lab ChuangXin).
Methodology: 20 cucumbers and 3 replicates were determined for growth parameters. The determination of total soluble sugar and soluble protein content are the methods of Anthrone

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colorimetry and Coomassic Brilliant Blue. Chlorophyll content was tested by using ethanol immersion extraction method. Gas exchange parameters were measured with LI-6400XT portable photosynthesis system. Chlorophyll fluorescence parameters of leaves were measured by a Handy PEA Fluorometer.

Results: Our results showed that 50% saturation HRW significantly enhanced the growth and development of cucumber seedlings, including the improvement of fresh weight, plant height, stem diameter and leaf area. These responses were consistent with a significant increase of leaf water content, total soluble sugar content and soluble protein content, which was further confirmed by the determination of photosynthetic related parameters. Also, research results illustrated that HRW up-regulated chlorophyll content and changed chlorophyll fluorescence parameters of leaves. The increase of chlorophyll content promoted the absorption of light and enhanced plant photosynthesis. Furthermore, HRW changed the leaf stomata conductance and decreased transpiration so as to improve the photosynthetic rate.

Conclusion: Taken together, these results suggest that the effect of HRW on cucumber seedling was associated with plant photosynthesis. Therefore, the application of HRW may be a promising strategy to improve cucumber growth.

Keywords: Cucumber; hydrogen-rich water; growth and development; chlorophyll; gas exchange parameter.

1. INTRODUCTION

Cucumber (*Cucumis sativus*) is cucurbitaceae plant and a widely grown vegetable that originated in the Himalayan tropical rainforest area [1-2]. Indeed, China is the world's largest country of cucumber planting area. Because of weak regeneration, shallow root, poor resistance, and especially in the germination and seedling stage, when subjected to environmental stress, the cucumber growth and development will be significantly inhibited, seriously affecting the yield and quality of cucumber [3-5]. Therefore, it is necessary to improve the growth status of cucumber seedlings as well as the body’s resistance.

Hydrogen gas is colourless, tasteless, odourless, which is generally regarded as physiologically inert in hyperbaric medicine [6-7]. Recent studies revealed that hydrogen has potential as an antioxidant in preventive and therapeutic function in animal [8-12].

More recently, there has been a renewed interest that hydrogen has an active effect on plant physiology. However, as early as 50 years ago, it was found that exogenous hydrogen has the role of stimulating seed germination [13]. And, in the last few years, studies indicated that low concentration of hydrogen-rich water (HRW) can promote the elongation of root and stem of mung bean (*Vigna*) and delay fruit post-harvest maturity [14-15]. Indeed, there is evidence suggesting that HRW-induced adventitious root development in a heme oxygenase-1/carbon monoxide-dependent manner [16] and some researchers found nitric oxide (NO) is involved in hydrogen gas-induced cell cycle activation during adventitious root formation in cucumber [17-18]. Besides that, there are results revealing the involvement of H_2 in auxin-induced lateral root formation via NO signalling [19]. Furthermore, it has been reported that hydrogen-treated soil can increase cucumber seedling stem diameter, plant height, and chlorophyll content [20].

Hydrogen can repair plant oxidative damage through removing excess reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes. Hydrogen also plays a signalling molecule role in stress-plant resistant networks. These stresses including salt, heavy metal ions, high light and UV irradiation, pesticide and so on [21-26].

Under salinity stress, HRW can enhance rice and *Arabidopsis* (*Arabidopsis thaliana*) tolerance by increasing antioxidant defence and improving the expression of hydrogenase gene [14, 27]. In addition, HRW promotes the regulation of plant responses against heavy metal ions stress in Chinese cabbage (*Brassica campestris*) or alfalfa (*Medicago sativa*) by reducing ions uptake and decreasing ROS content [22, 28-31]. On the condition of high light and ultraviolet radiation stress, HRW inhibits the accumulation of O_2^- and H_2O_2 in the plant body and elevates the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) [23-24]. Besides, HRW can activate the expression and activity of heme oxygenase 1 to increase the tolerance of alfalfa to oxidative damage caused by paraquat [21]. Moreover, studies have shown that HRW affects the growth, metabolism and stress resistance of fungi.

In this report, cucumber seedlings were cultured in the matrix nutrient bowl, and treated with different saturation hydrogen-rich water. We investigated the seedling's physiological and biochemical changes induced by different saturation HRW, including growth parameters, chlorophyll content, net photosynthetic rate, photosynthetic parameters and so on. These results suggest a positive role of HRW in promoting cucumber seedling growth and development.

2. MATERIALS AND METHODS

2.1 Preparation of HRW

Hydrogen gas generator (provided by Shenyang Yixin Health Service Hydrogen Technology Co., Ltd.) was soaked into 2 L distilled water for 5 h to generate purified hydrogen gas. Then, HRW was immediately diluted to the required concentrations. Distilled water was taken as control.

2.2 Plant Material and Growth Conditions

The cucumber (C. sativus) used in this study was JinYan4 that was produced by Tianjin Hongfeng Vegetable Research Co., Ltd. (Tianjin, China). Cucumber seeds were sterilised in sterile water for 10 min at 55℃ and then were soaked in water for 3 h at room temperature and germinated in the darkness at 27 ± 1℃ for 24 h. The seeds of white tip were sown in pots with a nutrient compound of peat soil-vermiculite (1:2, v/v) under greenhouse conditions for two weeks. 14-day-old cucumber seedlings were treated with different saturation hydrogen-rich water in root for three times every three day. Each group was treated with 20 plants and 3 replicates. After 24 h of the last treatment, growth parameters were determined and plant tissues were sampled immediately.

2.3 Determination of Fresh Weight, Plant Height, Leaf Area and Stem Diameter

Growth tests were repeated three times with 6 plants each. Plant height and fresh weight were determined by tape (FM 566X, China) and electronic balance (LE2O4E/02, China) [32]. The leaf areas were measured following paper weighing method [33], and the stem diameter was measured by vernier caliper (Threecircle, China) [32].

2.4 Analysis of Leaf Water Content and Root Water Content

The water content was measured following Li et al. [34]. And the water content of leaf and root were calculated as follow: [(FW-DW) / FW] × 100%, where FW and DW represent the sample fresh weight and dry weight, respectively.

2.5 Determination of Total Soluble Sugar and Soluble Protein Content

Dry root tissues (0.1 g) were homogenised in 10 mL 80% ethanol solution. The content of total soluble sugar was determined according to Zhang et al. [35]. The soluble protein content was measured using Coomassic Brilliant Blue method [36].

2.6 Chlorophyll Content of Leaves

Fresh leaves (0.2g) were grinded with 80 % acetone until white, after static 5 min，filter and add volume to 25 mL. The OD value of the solution at wavelengths of 665 nm, 649 nm and 470 nm was measured by ultraviolet spectrophotometer, and calculated chlorophyll content [37].

2.7 Effects of HRW on Gas Exchange Parameters

The net photosynthetic rate (Pn), stomata conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) were measured at 9:00 am to 11:30 am with LI-6400XT portable photosynthesis system (Li-6400, American). The air temperature, relative humidity, CO_2 concentration, and photosynthetic photon flux density (PPFD) were maintained at 25℃, 85%, 360 $\mu L \cdot L^{-1}$, and 800 mol $m^{-2}s^{-1}$, respectively.

2.8 Chlorophyll Fluorescence Parameters of Leaves

Chlorophyll fluorescence parameters of leaves were measured by a Handy PEA Fluorometer (Hansatech, Kings Lynn, UK). Plants were kept in the darkness for 30 min before measurement.

2.9 Statistical Analysis

Statistical significance was determined by *t*-test using SPSS statistical 17.0 software (P < 0.05) [38]. Plots were constructed by Origin 8.5 software.

3. RESULTS

3.1 HRW Promoted Cucumber Seedling Growth

The results showed that different saturation HRW (0, 20, 50 and 100%, respectively) treated cucumber exhibited different promote effect. A maximal inducible response was observed in 50% HRW-treated plants (Table 1). Compared with control cucumber, fresh weight and plant height of 50% HRW-treated seedlings increased 39.86% and 31.17%, respectively. As shown in Table 1, leaf area of 50% HRW-treated and 100% HRW-treated cucumber increased 23.25 and 17.29 cm^2, respectively. And treatment with 50% HRW and 100% HRW increased cucumber seedlings stem diameter by 22.03% and 28.81% compared with control group, respectively. However, 20% HRW had no effect on leaf area and stem diameter.

3.2 HRW Regulated Leaf Water Content and Root Water Content

The results of Fig. 1 revealed that leaf water content was influenced by the HRW treatment, the increase being 8.50%, 7.75% and 6.92% in 20%, 50% and 100% HRW groups, respectively. However, the highest root water content was seen in the control, as the hydrogen saturation increase, the root water content decreased significantly.

Table 1. Cucumber seedling growth parameters after treatment with different saturation hydrogen-rich water (HRW)

HRW	Fresh weight (g)	Plant height (cm)	Leaf area (cm^2)	Stem diameter (cm)
Control	$16.66±1.71^{c}$	$26.50 ±1.94^{c}$	$63.38±5.35^{c}$	$0.59±0.02^{b}$
20%	$21.46±2.4^{bc}$	$32.27±1.20^{bc}$	$70.93±5.19^{bc}$	$0.57±002^{b}$
50%	$27.70±2.06^{a}$	$38.96±3.25^{a}$	$86.63±6.07^{a}$	$0.72±0.03^{a}$
100%	$24.20±1.41^{ab}$	$32.53±0.91^{b}$	$80.67±5.42^{ab}$	$0.76±0.02^{a}$

Data are presented as means ± SE; values followed by the same letter do not differ significantly at P < 0.05 by Duncan test

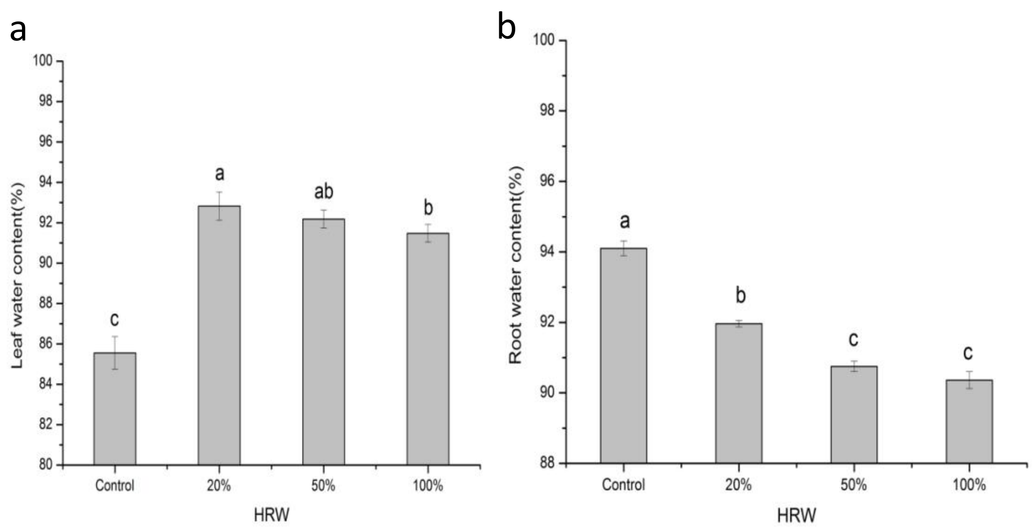

Fig. 1. Leaf water content (a) and root water content (b) of cucumber seedlings after the treatment with different saturation hydrogen-rich water (HRW). Data are means ± SE from three independent experiments. Bars with different letters are significantly different at *P* < 0.05 according to Duncan's multiple range test

3.3 HRW Increased Total Soluble Sugar Content and Soluble Protein Content

After treated with different saturation HRW, the cucumber root total soluble sugar content was up-regulated with the increasing of hydrogen saturation, and they were respectively increased 67.13%, 79.90%, 120.84% compared with control. It can be speculated that hydrogen is helpful for the formation of soluble sugar and may be a dose-dependent. Meanwhile, the soluble protein content of 50% and 100% HRW groups was also significantly ($p<0.05$) higher than control groups, and the content were 1.95 and 1.71 times of control.

3.4 Effects of HRW on Chlorophyll Content and Photosynthetic Rate in Cucumber Seedling Leave

Compared with control group, 50% HRW group significantly increased chlorophyll content and chlorophyll a content, but chlorophyll b and chlorophyll a/b were not influenced by HRW.

The changes of Pn, Gs, Ci and Tr of cucumber seedling leaves are shown in Fig. 4. The Pn was significantly increased in the 50% HRW group and 100% HRW group compared to the control group, and the increase brought about being 17.02% and 14.24%, respectively. Meanwhile, the Gs of cucumber seedling leaves was significantly lowered in the 50% and 100% HRW groups. Furthermore, compared with the control group, Tr was also significantly decreased in the 20%, 50% and 100% HRW groups. However, the Ci of cucumber seedling leaves had no difference.

3.5 Effect of HRW on Chlorophyll Fluorescence Parameters in Cucumber Seedling Leave

Minimal fluorescence (F0), maximal fluorescence (Fm) and intrinsic PS Ⅱ efficiency (Fv/Fm) were influenced by the 50% HRW in cucumber seedlings, the decrease being 6.47%, 12.43% and 2.16%, respectively. However, neither 20% HRW group nor 100% HRW group significantly affected F0, Fm and Fv/Fm, compared with control group (Fig. 5).

4. DISCUSSION

Cucumber is an important vegetable crop in the world. It is also one of the main vegetable crops with wide planting areas and large cultivated areas in China. It has the characteristics of high yield, abundant nutrition and good benefit. However, cucumber is very sensitive to the environment because of the shallow distribution of cucumber roots and weak regeneration. The current study indicated a significant improvement in the fresh weight, plant height, photosynthetic pigment content and net photosynthetic rate of cucumber seedlings treated with HRW, and 50% HRW is most effective.

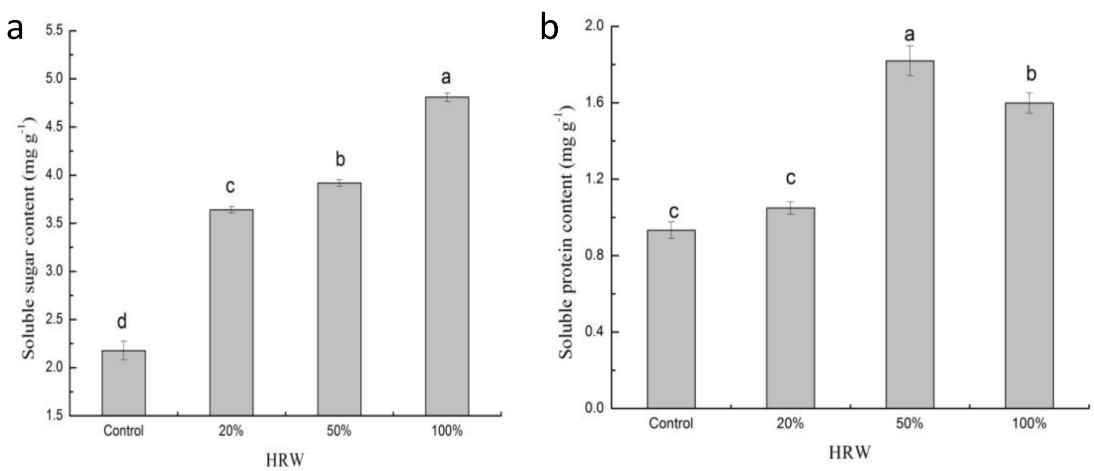

Fig. 2. Soluble sugar (a) and soluble protein (b) of cucumber seedlings were measured after the treatment with different saturation hydrogen-rich water (HRW). Data are means ± SE from three independent experiments. Bars with different letters are significantly different at *P* < 0.05 according to Duncan's multiple range test

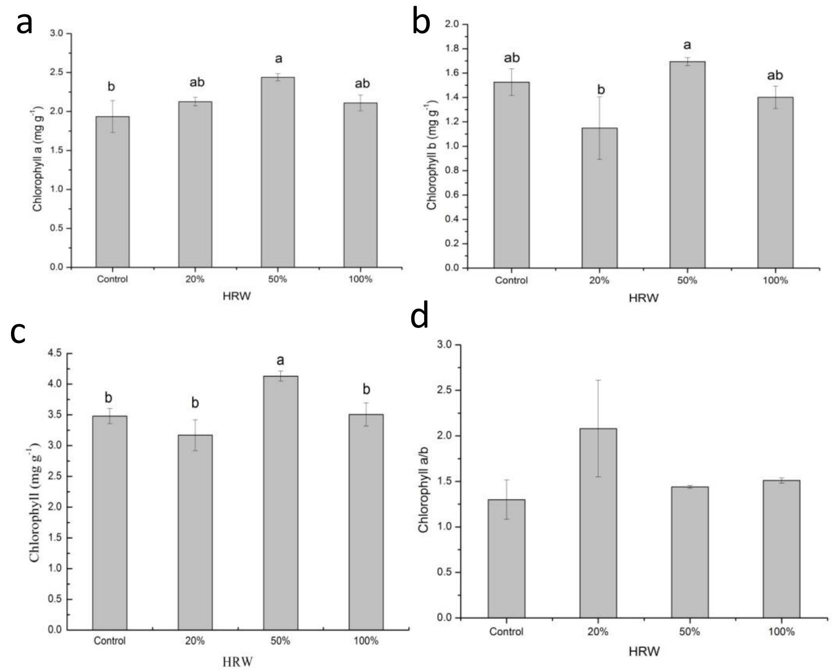

Fig. 3. Chlorophyll a (a), Chlorophyll b (b), Chlorophyll (c) content and Chlorophyll a/b (d) in cucumber leaf after the treatment with different saturation hydrogen-rich water (HRW). Data are means ± SE from three independent experiments. Bars with different letters are significantly different at *P* < 0.05 according to Duncan's multiple range test

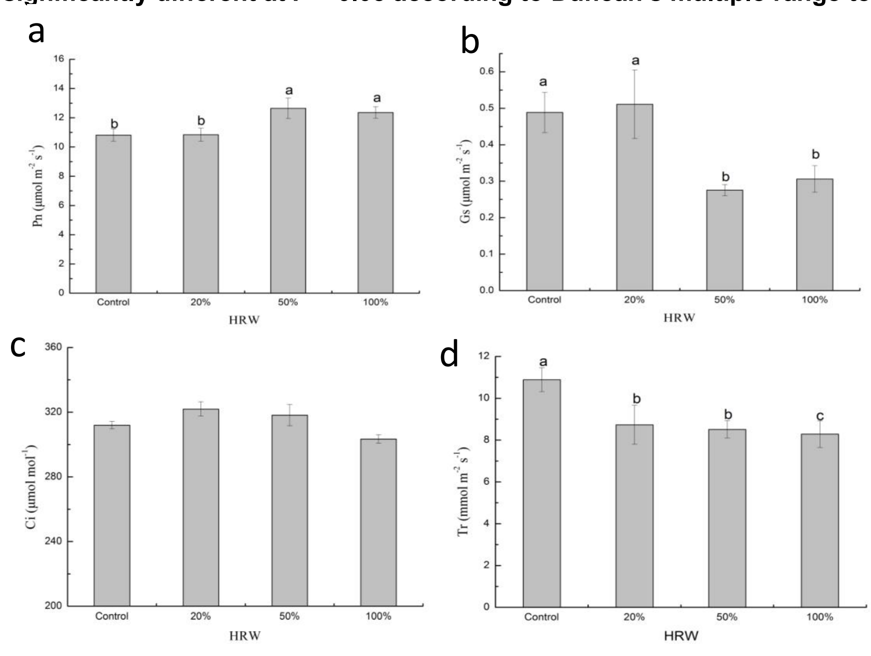

Fig. 4. Effects of different saturation hydrogen-rich water (HRW) on the net photosynthetic rate (Pn) (a), stomata conductance (Gs) (b), intercellular CO_2 concentration (Ci) (c) and transpiration rate (Tr) (d) of Cucumber Seedlings. Data are means ± SE from three independent experiments. Bars with different letters are significantly different at *P* < 0.05 according to Duncan's multiple range test

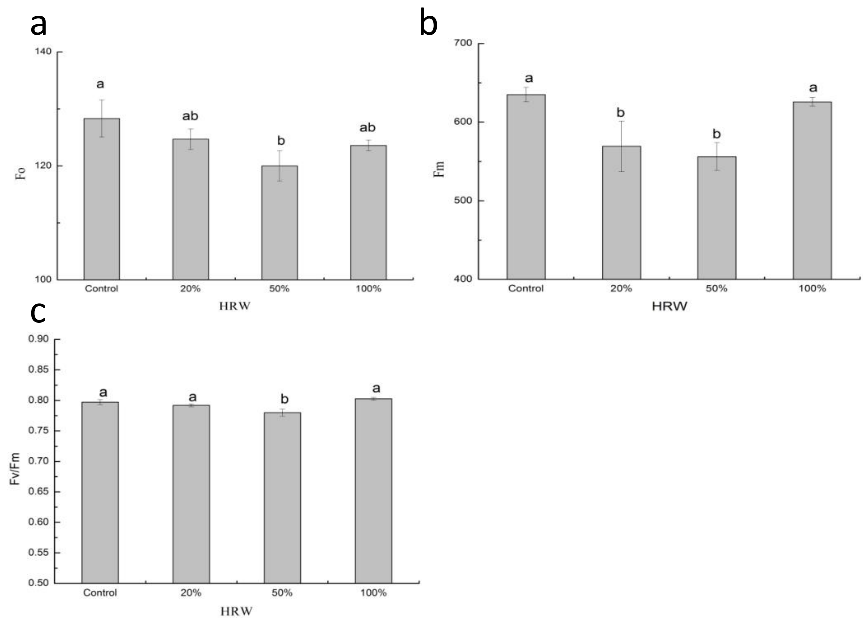

Fig. 5. Minimal fluorescence (F_0) (a), maximal fluorescence (F_m) (b) and intrinsic PS Ⅱ efficiency (F_v/F_m) (c) of cucumber seedlings after the treatment with different saturation hydrogen-rich water (HRW). Data are means ± SE from three independent experiments. Bars with different letters are significantly different at *P* < 0.05 according to Duncan's multiple range test

Recent studies have illustrated that hydrogen has become an important signaling molecules and maybe it can become an effective, simple, and economic therapeutic gas in the clinical trials [39-41]. Furthermore, hydrogen also acts as a novel and cytoprotective regulator in the improvement of plant tolerance, including drought, salt, heavy metals, UV irradiation tolerance [23,27-28,42]. Besides, hydrogen can indirectly enhance the plant's resistance to stress by affecting soil microbial composition [43]. Hydrogen released from nitrogen fixation could promote the growth of plant growth-promoting Rhizobacteria (PGPR), especially the growth of hydrogen-oxidising bacteria and the growth of rhizobia. This is the new theory of hydrogen fertiliser [44]. Although there are several reports discovering hydrogen has a positive effect on plant growth and resistance, little information was known about the exact mechanism and even physiological roles of hydrogen in plants.

In this paper, the work presented the physiological role in HRW-induced cucumber seedlings growth. We found that 50% and 100% HRW could significantly increase the fresh weight, plant height, stem diameter and leaf area of cucumber seedlings. That is consistent with the reports that HRW can promote alfalfa fresh quality and increase the fresh weight and root elongation of *Arabidopsis* seedlings [27, 29]. It also agrees with the study that 50% HRW increased Chinese cabbage seedlings fresh weight [31]. Zhao et al. [20] found hydrogen-treated soil can increase cucumber seedling stem diameter, plant height. Furthermore, HRW could improve plant growth under stress. For example, HRW treatment could alleviate the inhibition of paraquat and improve seedling's root growth [42].

HRW could regulated leaf water content and root water content of cucumber seedlings. 50% HRW treated seedlings had the highest leaf water content (Fig. 1a) compared with other treatment group. 50% HRW seedling's high photosynthetic rate consists with its high-water content. From other studies we know nutrient of nitrogen and exogenous *Rhizophagus irregularis* have positive effects on relative water content [25]. We believed that the high-water content of cucumber leaves was beneficial to the photosynthesis of plants and promoted the metabolism of cucumber seedlings. Also, the experimental results (Fig. 1b) showed that HRW may promote the absorption of water from the roots to the leaves.

Soluble sugar can be used as a penetrating substance involved in the plants stress resistance. The higher the sugar content is, the higher the plant metabolism is. With the increase of hydrogen saturation, the root soluble sugar content also increased in a dose-dependent manner (Fig. 2). The increase of soluble sugar by HRW was also observed in rice during seed germination [22]. Soluble protein content was positively correlated with plant resistance. In the present study, HRW promoted the soluble protein content of cucumber seedlings, and the increase of soluble protein content may be related to the synthesis and metabolism of resistance proteins and defense enzymes in plants. The same results were observed by [28]. They reported that HRW could alleviate cadmium toxicity in *Medicago sativa* accompanied by an increased protein content.

Chlorophyll content is an important factor affecting photosynthesis light energy absorption [45]. Its content, to a certain extent, can reflect the ability of plant assimilation of substances. A small number function of chlorophyll a is mainly to converge the light energy into chemical energy for photochemical reactions, and chlorophyll b is mainly to collect light energy. Maintain a relatively high chlorophyll a content in the body, which can ensure the full utilisation of the light energy of the plant and improve the conversion rate [46-47]. 50% HRW could increase chlorophyll a content (Fig. 3a) and chlorophyll content (Fig. 3c). This is consistent with the results of Xie et al. [27] and Zhao et al. [20]. The increased chlorophyll content and chlorophyll a content of cucumber leaves indicated that treatment with 50% HRW made the cucumber leaves more easily capture and convert light energy, which played an important role in improving photosynthetic rate. The higher chlorophyll content is, as well as the higher cucumber seedlings net photosynthetic rate is (Fig. 4a).

Stomata is the main organ on plant leaves, controlling the absorption of CO_2 in plant photosynthesis and the transport of H_2O in transpiration, so the stomata have an important decisive effect on crop photosynthetic physiology. The present data demonstrated that HRW treatment resulted a significant increase in the photosynthetic rate. Gs and Tr were significantly lower in the 50% HRW group than in the control group. Coincidentally, these results accord with its high-water content (Fig. 1). However, Ci had no difference with control. The results agreed with Zhang et al. [24] that exogenous 50% HRW treatment stimulated maize seedlings plant height and net photosynthetic rate. Also, the effect of HRW is similar to Ogweno et al. [48] findings that the 24-epibrassinolide can improve tomato Pn and dry weight. Due to the smaller Gs of cucumber seedlings with 50% HRW and 100% HRW, the water loss caused by transpiration was reduced. Therefore, it can be speculated that the change of stomata conductance may be one of important reasons for the increase of photosynthesis rate in HRW-induced cucumber seedlings. Xie et al. [49] reported that HRW can improve *Arabidopsis* drought resistance by promoting stomatal closure. Only Ci with the simultaneous decline in the case of Gs can explain that the Pn decline is caused by the stomata limitation [50]. In this experiment, Gs of 50% HRW group was lower than 100% HRW group, but 50% HRW group had a higher Ci than 100%group. Therefore, HRW enhanced the photosynthetic rate of cucumber seedlings not only by improving the stomata conductance but also adjusting the non-stomata factors.

Chlorophyll fluorescence can be used as a good probe for in vitro detection of the activity and function of PS Ⅱ reaction center in plant leaves [51]. In general, the chlorophyll fluorescence and photosynthetic rate are negatively correlated with each other. The current study demonstrated a significant decrease in F0, Fm and Fv/Fm of cucumber seedlings treated with HRW, which indicated that the ratio of the light absorbed by the pigment of the cucumber leaves was reduced in the form of fluorescence, and the electron transfer through PS Ⅱ increased.

5. CONCLUSION

The results of this research suggest that HRW can promote cucumber growth by heightening photosynthetic rate, meanwhile the treatment of 50% HRW was the most effective. The treatment of HRW caused an apparent increase in chlorophyll content, while the chlorophyll fluorescence parameters declined. That demonstrated that HRW may enhance the photosynthetic rate of cucumber seedlings by increasing the chlorophyll content and maintaining efficient light absorption, transmission and conversion. As a result of that, there was an increase in fresh weight, plant height, stem diameter and leaf area. Additionally, total soluble sugar content and soluble protein content were also obviously up-regulated, which suggested that HRW may reinforce the plants resistance against external stress. Therefore, the application of HRW can promote the growth and improve physiology feature of cucumber seedlings. Due to characteristics of easy to make, green and economical, HRW may contribute to raising production and quality, as well as reducing pesticides and chemical pollution and other aspects of great significance.

ACKNOWLEDGEMENT

This work was supported by The National Key Research and Development Program of China under Grant 2016YFD0201004.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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