Effect of silicon on photosynthetic rate and the chlorophyll fluorescence parameters at hydroponically grown cucumber plants under salinity stress

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ABSTRACT

The present research aims at evaluating the effect of silicon on the photosynthetic rate and the chlorophyll fluorescence parameters of hydroponically grown young cucumber plants (Cucumis sativus L.), cv. Gergana, under salinity stress. The experiment was conducted in a climatic chamber at the Department of Plant Physiology and Biochemistry, Agricultural University – Plovdiv, Bulgaria. Four variants were examined: 1 – control, 2 – NaCl, 3 – Si, and 4 – NaCl + Si. Plants were grown in a nutrient solution and were treated with 50 mM NaCl and 1.5 mM Si in form of Na$_2$SiO$_3$. The results showed a decrease in the photosynthetic rate by 25% for the NaCl variant compared to the control. Silicon supply increased the photosynthetic rate of the salt-stressed plants by 17%. Salinity had also a negative effect on the chlorophyll fluorescence parameters and the content of photosynthetic pigments, which were dramatically reduced. It was established that the silicon supply has a positive influence on the photosynthetic rate, the chlorophyll fluorescence parameters and the pigment content of the treated cucumber plants. The content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids increased.

Keywords: abiotic stress, chlorophyll fluorescence, cucumber, photosynthesis, salinity, silicon

Целта на изследването е да се оцени ефектът на силиций върху скоростта на фотосинтезата и параметрите на хлорофилната флуоресценция на млади растения краставица (Cucumis sativus L.), сорт Гергана в условия на солеви стрес. Експериментът е проведен в климатична камина в катедра "Физиология на растенията и биохимия", Аграрен университет – Пловдив, България. Изследвани са четири варианта, състоящи се от: 1 – контрола, 2 – NaCl, 3 – Si и 4 – NaCl + Si. Растенията са отглеждани в хранителен разтвор и са третирани с 50 мМ NaCl и 1.5 мМ Si под формата на Na$_2$SiO$_3$. Резултатите показват понижение в стойността на PN с 25% при третираните с NaCl растения в сравнение с контролата. Прилагането на Si увеличава скоростта на PN на растенията, подложени на засоляване със 17%. Солевото третиране оказва отрицателен ефект и върху параметрите на хлорофилната флуоресценция и съдържанието на фотосинтетичните пигменти, които са силно редуцирани. Установено е, че приложението на силиций действа благотворно на скоростта на фотосинтезата, параметрите на хлорофилната флуоресценция и съдържанието на фотосинтетични пигменти. Отчетено е повишено съдържание на хлорофил a, хлорофил b, общ хлорофил и каротеноиди.

Ключови думи: абийотичен стрес, краставица, пигменти, силиций, фотосинтеза, хлорофилна флуоресценция
INTRODUCTION

Silicon (Si) is one of the most common elements in the Earth’s crust. Most of the element is present in the soil in the form of insoluble oxides and silicates. Nevertheless, it can also be seen in water-soluble forms. Si is the only element which does not damage plants when it is accumulated in excess due to its non-dissociative properties at physiological pH and polymerization (Mahdieh et al., 2015). Many studies have shown that monocotyledonous plants, especially the members of Poaceae family, respond positively to the increased content of silicon in the nutrient medium (Gong et al., 2006; Shi et al., 2013). The influence of silicon on growth-enhancing and plant-resistance metabolic processes is recorded under stress conditions mainly. Studies have been conducted in various methodological settings. They have shown that silicon is able to reduce the effect of stress caused by abiotic environmental factors (salinization, drought, waterlogging, heavy metal pollution, nutritional imbalance, elevated levels of radiation, injury and frost) and biotic factors (plant pests and diseases).

The positive effect of silicon application has been proven also upon some dicotyledonous crops, especially when plants are subjected to biotic or abiotic stress. There have been studies on the effect of silicon on stimulating growth and productivity in cucumber, zucchini, beans, tomato, etc. in conditions of abiotic stress, including salinization (Zhu et al., 2004; Zuccarini et al., 2008; Harizanova et al., 2014).

Various studies have confirmed that salinity provokes photoinhibition and photo-damages in Photosystem II (PSII) and reduces its activity (Misra et al., 2006). The decrease in photosynthetic capacity in saline conditions can be due to various disturbances: reduced stomatal conductance; inhibition of chlorophyll biosynthesis; reduction of photosynthetic electron transport; suppression of biochemical processes and/or direct damage of the photosynthetic apparatus (Tavakkoli et al., 2010).

The influence of silicon on many stress-promoting and growth-enhancing metabolic processes has not been yet well studied (Liang et al., 2007; Guntzer et al., 2012; Gonzalo et al., 2013). Chen et al. (2011) observed the basic quantum yield ($F_v/F_m$) and maximum quantum efficacy ($F_v/F_m$) of the photochemistry of PSII in plants exposed to drought stress and treated with silicon. There are speculations about the mechanism of silicon protection. The positive effect of silicon on photosynthesis could be either due to its protective role on chloroplasts, or to the increased concentration of photosynthetic pigments responsible for the light absorption, or both. According to Liang et al. (2003), the treatment of salt-stressed barley with Si reduces damage to the ultrastructure of chloroplasts by protecting the double membranes and maintaining the integrity of the granae.

The aim of the current work is to study the effect of silicon on photosynthesis and chlorophyll fluorescence in hydroponically grown cucumber plants under salinity stress.

MATERIALS AND METHODS

Laboratory tests conducted to study the physiological effect of Si in hydroponically grown cucumbers in saline conditions were carried out in phytostatic chambers at the Department of Plant Physiology and Biochemistry, Agricultural University – Plovdiv. Plants were grown hydroponically under controlled environmental conditions: photoperiod 14/10 hours (light/dark), light intensity (PAR) - 250 μmol/m²/s, air temperature 24±2 °C/17±2 °C (day/night) and relative air humidity 65±5%. The seeds were placed for germination in Petri dishes on filter paper soaked with distilled water for 24 hours in a thermostat. The germinated seeds were transferred to plastic terrains with inert material (perlite) for a period of 5-7 days. After that procedure, plants at the same developmental phase were selected and transferred to plastic containers with nutrient solution. The experimental design consisted of 4 variants: 1 - control plants; 2 - plants treated with 50 mM NaCl, added to the nutrient solution; 3 - plants treated with 1.5 mM Si, added to the nutrient solution; 4 - plants treated with 50 mM NaCl and 1.5 mM Si, added to the nutrient solution. Each variant was replicated 3 times. Cucumber plants of all variants were
grown hydroponically in a nutrient solution containing all the necessary macroelements (KNO$_3$ - 5 mM; Ca(NO$_3$)$_2$ - 1.5 mM; NH$_4$H$_2$PO$_4$ - 1 mM; MgSO$_4$ - 1 mM); and trace elements (50 μM KCl, 10 μM ZnSO$_4$, 1.6 μM CuSO$_4$, 0.1 μM Na$_2$MoO$_4$, 0.4 μM MnSO$_4$, 12.5 μM H$_3$BO$_3$, and Fe-EDTA (96 μM). The pH solution was adjusted to 6-6.5 by adding KOH and HCl. The solution was replaced once a week and daily aerated. Ten days after germination, which coincided with the appearance of a third true leaf, the plants were treated with NaCl and Si for 10 days. All analyses were made at the end of the treatment period.

**Analysis**

**Rate of net photosynthesis**

The rate of net photosynthesis ($P_N$) was determined with LCpro + portable photosynthetic system (Analytical Development Company Ltd., Hoddesdon, England). Measurements were performed under the following conditions: Photosynthetically active radiation (PAR) of 500 μmol/m$^2$/s, temperature 25 °C and natural external CO$_2$ concentration of about 400 vpm.

**Content of photosynthetic pigments**

The content of photosynthetic pigments was determined spectrophotometrically by the Lichtenthaler method (1987) and was expressed as mg/g fresh leaf material.

**Chlorophyll fluorescence**

The main parameters of chlorophyll fluorescence were determined by MINI-PAM impulse modulating fluorimetry (H. Walz, Effeltrich, Germany) in intact leaves of cucumber plants after dark adaptation. The minimum chlorophyll fluorescence ($F_{0}$) was determined after 60 minutes of leaf dark adaptation by incorporating a measuring light with an intensity of 0.02-0.2 μmol/m$^2$/s. Immediately afterward, maximum fluorescence was determined in dark-adapted leaves ($F_{m}$) by incorporating a light saturation pulse with an intensity of 5,500 μmol/m$^2$/s and a duration of 0.8 s. Variable fluorescence ($F_v$) was calculated automatically by the difference between the maximum and minimum fluorescence. The maximum efficiency of Photosystem II was calculated according to Schreiber (2004).

**Statistical data processing**

The data were presented as mean ± SD. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis, as well as Duncan's comparative method with a validity of differences determined at a 95% significance level.

**RESULTS AND DISCUSSION**

Salt stress caused a reduction in cucumber plant growth. Growth analysis showed that the net assimilation rate (NAR) – one of the components of the plant relative growth rate was strongly affected by the salt treatment (Figure 1). This “physiological” component – (NAR) of the salt-stressed plants was reduced by 34% compared to the control. The addition of silicon definitely had a positive effect on the stressed plants. The treated plants decreased the value of that parameter only by 11% (Figure 1).

Net assimilation rate is related to the photosynthesis, the respiration activity and the relative volume of plant organs, which do not photosynthesize. All of them were also negatively affected by NaCl (data not presented) but the Si supply had a beneficial effect.

Results of the net photosynthetic rate analysis showed a strong effect of the salinization (Figure 2). The assimilation capacity of NaCl treated plants was reduced by 25%. The addition of silicon enhanced the photosynthetic activity of stressed plants and the decrease relative to the control was lower (17%). For plants treated with Si only, there was no statistically proven change in the value of the indicator (Figure 2).

One of the most important factors for the photosynthetic activity appeared to be the pigments. The content of photosynthetic pigments and their ratio is given in Table 1. Results showed a dramatic decrease in the content of chlorophyll a, chlorophyll b, and carotenoids (by about 50% with comparison to the control). The ratio of chlorophyll a/b was lowest in the salt-treated variant,
and the ratio of chlorophylls/carotenoids did not change significantly compared to the control plants because of the decrease of both types of the pigments. Silicon added to the salt-stressed plants nutrient solution contributed to a significantly lower reduction in the content of photosynthetic pigments (less than 20% relative to the control), and when applied alone - approximates the value of control plants' pigments.

The other factor which characterized the photosynthetic activity is chlorophyll fluorescence. It gives information about PSII activity in response to environmental factors. Chlorophyll fluorescence in dark-adapted leaves (Table 2) varied slightly between all variants (2 to 15% in comparison to the control). Salt treatment caused an increase in minimum fluorescence ($F_0$) by 9%, while the maximum and variable fluorescence remained
Table 1. Content and ratio of photosynthetic pigments (mg/g fresh weight) in cucumber leaves (Cucumis sativus L.), cv. Gergana, after 10 days treatment with NaCl and Si

<table>
<thead>
<tr>
<th>Variant</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl (a+b)</th>
<th>Carotenoids</th>
<th>Chl a/b</th>
<th>Chl (a+b)/carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.05a</td>
<td>0.74a</td>
<td>2.78a</td>
<td>0.67a</td>
<td>2.77a</td>
<td>4.14a</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.99d</td>
<td>0.47c</td>
<td>1.46d</td>
<td>0.35c</td>
<td>2.1c</td>
<td>4.15c</td>
</tr>
<tr>
<td>Si</td>
<td>1.92b</td>
<td>0.63b</td>
<td>2.54b</td>
<td>0.65b</td>
<td>3.08b</td>
<td>3.88b</td>
</tr>
<tr>
<td>NaCl+Si</td>
<td>1.69c</td>
<td>0.61b</td>
<td>2.29c</td>
<td>0.58b</td>
<td>2.77b</td>
<td>3.97bc</td>
</tr>
</tbody>
</table>

The data are presented as an average of 3 replicates. a, b, c, d Different letters show statistically proven differences between the variants (P<0.05). Chl – chlorophyll.

Table 2. Parameters of chlorophyll fluorescence in dark-adapted cucumber leaves (Cucumis sativus L.), cv. Gergana, after 10 days' treatment with NaCl and Si

<table>
<thead>
<tr>
<th>Variant</th>
<th>(F_0)</th>
<th>(F_m)</th>
<th>(F_v)</th>
<th>(F_v/F_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>268b</td>
<td>1.411b</td>
<td>1.143b</td>
<td>0.81a</td>
</tr>
<tr>
<td>NaCl</td>
<td>293ab</td>
<td>1.413b</td>
<td>1.120b</td>
<td>0.792a</td>
</tr>
<tr>
<td>Si</td>
<td>307b</td>
<td>1.567b</td>
<td>1.260b</td>
<td>0.804ab</td>
</tr>
<tr>
<td>NaCl+Si</td>
<td>294ab</td>
<td>1.484ab</td>
<td>1.190ab</td>
<td>0.802ab</td>
</tr>
</tbody>
</table>

The data are presented as an average of 3 replicates. a, b Different letters show statistically proven differences between the variants (P<0.05). 1 \(F_0\) – minimum fluorescence; 2 \(F_m\) – maximum fluorescence; 3 \(F_v\) – variable fluorescence; 4 \(F_v/F_m\) – maximum photochemical efficiency of PSII.

unchanged. With regard to the maximum photochemical efficiency of PSII expressed by the \(F_v/F_m\) ratio, very low inhibition of the parameter was observed.

Silicon applied to the nutrient solution of the salt-stressed plants caused an increase in \(F_0\), \(F_v\), and \(F_v/F_m\) compared to the variant treated only with NaCl. Applied in non-stress conditions, silicon caused a slight increase in the values of minimum, maximum and variable fluorescence, but did not affect the \(F_v/F_m\) ratio.

Salinity is one of the major abiotic factors of the environment, affecting negatively the growth and development of plants (Khadri et al., 2007). A number of studies have shown a negative correlation between photosynthetic activity and salinization (Romero-Aranda et al., 2006; Silva et al., 2008; Gama et al., 2009). According to Renault et al. (2001) and Gama et al. (2009), salinity-induced inhibition of growth may result because of the suppression of photosynthesis.

Liu et al. (2015) studied the influence of silicon on sorghum plants in saline conditions. They observed an increase in the rate of net photosynthesis when silicon was added to the nutrient medium of stressed plants. According to the authors, the increase in photosynthetic rate and stomatal conductivity was a result of the increased hydration of tissues of Si-treated plants. Silicon is able to increase the rate of net photosynthesis in cucumber plants under conditions of other stress factors (Mn excess) and approximate its values to levels close to those of control plants (Feng et al., 2009).

According to Stepień and Klobus (2006) and Pagter et al. (2009), the decreased rate of photosynthesis may be due to both stomatal and non-stomatal constraints. In order to reduce water loss, salinity and induced osmotic stress provoked rapid changes associated with
occlusion (Silva et al., 2008). It is a well-known fact that carotenoids are an important component of PSI and PSII photo-protective mechanisms due to their participation in the quenching of the singlet oxygen state and the triplet state of chlorophyll (Jaleel et al., 2009). According to some authors (Barhoumi et al., 2007), the reduced content of photosynthetic pigments is due to both, their biosynthesis disturbances and increased destructive processes. Relationships between individual groups of pigments are widely used as to indicate the presence of plant stress (Pinheiro et al., 2008), as well as to early indicate the aging of tissue due to stress disorder.

The presented results were in agreement with the results obtained for cucumber (Ma et al., 2004; Feng et al., 2010), potatoes (Pilon et al., 2013), wheat (Tuna et al., 2008) and corn (Vaculík et al., 2015). It was proved that silicon reduced the degradation of chlorophyll in cucumber plants under stress conditions (Ma et al., 2004). Authors concluded that the first factor by which silicon influences the accumulation of dry mass was the increase in photosynthesis, and the second factor was the increase in water-holding capacity. According to Tuna et al. (2008), application of silicon to salt-stressed plants can restore the amount of chlorophyll to the control level. Feng et al. (2010) also reported a decreased content of chlorophyll a, chlorophyll b and carotenoids in the cucumber leaf exposed to excess Mn stress. By adding silicon to the nutrient medium of stressed plants, the content of photosynthetic pigments increased. The authors observed an increase in the concentration of pigments under the influence of silicon in non-stress conditions. Vaculík et al. (2015) reported that the ratio of chlorophyll a/b decreased under stress conditions.

The established changes in the content of photosynthetic pigments and their relationships can be seen not only as a result of the effects of salt stress and silicon but also as an adaptive mechanism. This reduces the proportion of absorbed light energy and protects the photosynthetic apparatus from energy recharge.

The possible speculation for the observed silicon stimulating effect is the increased N in tissues of the salinized plants (data not presented), which is an integral part of the chlorophyll molecule.

Chlorophyll fluorescence can be considered as an indirect indicator of the state of the integral photosynthetic process due to the functional relationship of PSII with the other components of the photosynthetic apparatus. This method is used to quickly and non-invasively assess the efficacy of quantum electron transport through PSII in the cucumber leaves, associated with CO₂ assimilation (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004). Chen et al. (2011) reported that in drought-stressed and silicon-treated plants, the maximum quantum efficacy (Fv/Fm) of PSII increased. According to the authors, the positive effect of silicon on photosynthesis is due to either its protective role on the chloroplasts, or to the increased concentration of pigments, which is associated with more intense light absorption, or both.

Feng et al. (2009) also observed decreases in the ratio of Fv/Fm and in the quantum yield of PSII electron transport in excess of Mn in cucumber. These negative effects were overcome by treating stressed plants with silicon. Authors believe that the positive effect of silicon application is due to the activation of enzymes from the ascorbate-glutathione cycle, thereby reducing the accumulation of H₂O₂ and reducing the rate of lipid peroxidation in the chloroplasts themselves. In young maize plants, grown under Cd-stress conditions, Vaculík et al. (2015) also reported a decrease in photochemical quantum yield, which was overcome, when the stressed plants were treated with silicon.

CONCLUSIONS

The research results show that silicon supply to the nutrient solution of salt-stressed cucumber plants in the early stages of their growth is capable to ameliorate the detrimental effect of salinity. The beneficial effect of silicon is attributed to the increased content of photosynthetic pigments, the improved parameters of chlorophyll fluorescence presented by the enhanced efficiency of PSII and the accelerated rate of net photosynthesis.
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