Changes in Photosynthetic Capacity of Cucumber Seedlings in Response to Different Nitrogen Supply

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***Abstract*—Using adequate nitrogen (N) supply is one of the main goals of sustainable plant production. Excessive application leads to health, environmental and economic problems. Horticultural systems are more sensitive but beside the question of sustainable agriculture, the demand for N fertilizers remains strong. Being 'green', but satisfied, we need to know the exact amount of N, which is sufficient and no more, or need to find efficient genotypes for adequate N use efficiency. The objectives of this research were to investigate photosynthetic capacity of cucumber (*Cucumis sativus* L cv. Rajnai fürtös) seedlings under different amount of N supply (5 times, half times, tenth of optimal N in the nutrient solution) in hydroponic conditions. The dry weight of root and shoot, the relative chlorophyll content (SPAD value), photosynthetic pigments content and optimal photochemical activity (Fv/Fm) were measured. The half reduction of optimal N supply did not reduce our measured parameters, the relatively large nitrogen deprivation did not cause significant differences compared to the optimal nitrogen supply.**

***Index Terms*—cucumber, nitrogen, SPAD value, chlorophyll, carotenoids, chlorophyll fluorescence**

I. Introduction

The main goal of sustainable agriculture is to feed the increasing global population and agree with heath-, environmental and economic criterions. The basis of successful plant production is the adequate nutrient supply. The intensive nutrient supply is crucial for horticultural plant systems, because they need to produce high amount with excellent quality on relative small fields. Nowadays horticultural systems are concentrated on quality in which the nutrient supply is fundamental. Using chemical nitrogen (N) fertilizers is the main tool for improving plant production. It is well known, that vegetables are sensitive for nitrate accumulation, which needs more cautions. Besides this dangerous nitrate accumulation – mainly for babies – nitrate, as a mobile ion, can leach from the soil and leads to environmental problems [1]. Use of excess nitrogen supply also can causes economic problems for agrarians.

The demand for higher amount for food with better quality is getting higher and higher. Nitrogen, often a limiting resource for plant growth as in cucumber [2], is required by plants in great quantities than any other mineral element. The main reason of reduced growth may have a declined stimulation of nitrate-reductase enzyme due to the insufficient N supply [3]. Nitrogen is the core constituent of a plant's nucleic acid, proteins, enzymes, and cell wall and pigment system [4]. Nitrogen nutrition influences the plant photosynthetic capacity through the decrease of synthesis of several key photosynthetic enzymes, especially of Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase), thus affecting the carbon assimilation, and subsequently also the photochemical processes in thylakoid membranes [5]. Rubisco is the most abundant protein on earth and contributes a high percentage to the total leaf nitrogen in C3 plants [6].

Besides of Rubisco, chlorophylls also contain relatively high portion of nitrogen. In addition to indicating plant nitrogen status, chlorophyll content is an important indicator of leaf senescence [7], and it can also be altered in response to environmental stresses [8]. Extraction of photosynthetic pigments from leaf tissue is useful quantitative and qualitative method for getting information about chlorophyll-a, -b and total carotenoids as well. Carotenoids have photoprotective role, mainly if the chlorophyll concentration is reduced by different environmental factors, like nutrient deficiency. In photosystem two (PSII), carotenoids can deactivate $^3Chl^*$ and $^1O_2{}^*$, and reduce reactive oxygen species formation due to the thermal dissipation of excess light energy because of nutrient deprivation [9], [10]. Nondestructive analysis of relative chlorophyll content with chlorophyll meters, for example the SPAD-502, provide a simple, quick, and nondestructive method for estimating leaf chlorophyll content [11], [12].

The chlorophyll fluorescence induction curve has several parameters for characterizing plant conditions. In dark adapted samples the value of Fv/Fm, as potential

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photochemical efficiency of photosystem two (PSII) estimates the maximum portion of absorbed quanta used in PSII reaction centers [13]. The indicator function of chlorophyll fluorescence is originated from the fact that fluorescence emission is complementary to alternative pathways of de-excitation which are primarily photochemistry and heat dissipation [14]. In healthy leaves, this value is always close to 0.8, independently of the plant species studied. A lower value indicates that a proportion of PSII reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants under stress conditions.

The objectives of this study were to determine the effects of different N supply – deficiency and excess – on the photosynthetic capacity of cucumber in early growth stage. Measuring several parameters which characterized plant production is a tool for evaluate the optimal nitrogen content for this genotype and help to find parameter to detect and catch insufficient N.

II. Materials and Methods

A. Materials

Cucumber (*Cucumis sativus* L cv. Rajnai fürtös) seedlings (24 days old) were used in our experiments under hydroponic conditions. The seeds were germinated on moistened filter paper at 25°C. After eight days the seedlings were transferred to a continuously aerated nutrient solution of the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 1μM H_3BO_3, 1μM $MnSO_4$, 0.25 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$. The H_3BO_3 concentrations were 10μM and iron was added to the nutrient solution as Fe-EDTA in a concentration of 10^{-4} M.

Three separated pots were set up in each treatment with 4 plants respectively. The following treatments were applied: optimal N content (control), 5 times of optimal N (5*N), half times of optimal N (1/2*N), tenth of optimal N (1/10*N) and no N in the nutrient solution (N-). The total N deprivation caused plants' death on the 10th day. The experiments were set up in the controlled environmental room. The light intensity was 300 $\mu molm^{-2}s^{-1}$, the day/night temperature was 25/20°C, the day/night time period was 16h/8h and the relative humidity was 65-75 %.

B. Methods

1) Dry weight determination of root and shoot

The actual dry weight of plant parts – root and shoot - was determined with thermal gravimetric analysis. The twenty four days old seedlings were separated to roots and shoots. Different parts were stored in the oven with 65°C for three days and measured the dry weight (g $shoot^{-1}$ and g $root^{-1}$) with analytical balance (OHAUS Explorer, Switzerland).

2) Determination of photosynthetic pigment contents

Chlorophyll-a, chlorophyll-b and total carotenoid contents were analyzed with UV/VIS spectrophotometer (Metertech SP-80, Taiwan). The extraction was prepared according to Moran and Porath (1989) [15]. The amount of chlorophyll-a, chlorophyll-b and total carotenoid content was calculated by Wellburne (1994) [16] formulas. For these measurements younger (4th) and older (1st) leaves were used in all treatments.

3) Relative chlorophyll content measurements (SPAD value)

The relative chlorophyll contents (SPAD value) of younger (4th) and older (1st) leaves were determined by relative chlorophyll meter (SPAD-502, Minolta, Japan). Three plants were measured per plots and each leaf the average value was calculated from 5 measurements.

4) Measurement of maximal photochemical activity (Fv/Fm) with the chlorophyll fluorescence method

The parameters of *in vivo* chlorophyll fluorescence were detected with a PAM 2001 (Walz, Germany) modulated light fluorometer as described by Schreiber *et al*. (1986) [17]. Samples were dark-adapted for 30 minutes. After dark adaptation, the initial fluorescence (F_0) was excited by weak light (0.1 $\mu molm^{-2}s^{-1}$). The maximal fluorescence (F_m) was induced by white saturating flash (8 000 $\mu molm^{-2}s^{-1}$). In this condition, QA, the first electron acceptor of PSII (photosystem two), is fully reduced. This allows the determination of the maximum quantum efficiency of photosystem II (PSII) primary photochemistry, given by Fv/Fm = (Fm-Fo)/Fm, as potential photochemical activity of leaves [13].

5) Statistical analysis

The number of replicates were 3-6. Analysis of Variance (ANOVA) was performed and using the Duncan's Multiple Range Test for mean separation. The data regarding the samples were presented as mean values±standard errors and analyzed using the t-test statistical analysis. The analysis of all data was conducted using SigmaPlot for Windows Version 12.0 (Systat Software Inc., Germany), with the significant level determined at 95% confidence limit ($p \leq 0.05$).

III. Result and Discussion

Due to the reduced N application generally reduce the growth of plants [18]. The dry weight of control shoot was 0.972 g (±0.216 g), and root 0.133 g (±0.046g) (Fig. 1). The excess nitrogen application (5*N) increased the shoot dry weight by 4%, and the half N deprivation (1/2*N) also increased it by 13%. The larger N deprivation (1/10*N) significantly reduced the shoot dry weight by 39%. The root dry weight declined with 10% by the effect of 5-times more nitrogen, compare to the control value. The nitrogen deprivation induced higher root dry weight by 19% in ½-N and by 59% in 1/10*N compared to the control. Nitrogen nutrition has significant effects on root and shoot relations [19]. Nitrogen deficiency increased root surface area and decreased shoot growth resulted in a higher root/shoot ratio, because increased consumption of assimilates and reduced the amount of nitrogen transported to shoot and resulted in an increased root and shoot ratio [19], [20], [21]. Nitrogen in excess causes an excessive shoot growth,

reduced the assimilate availability for root, and reduced the root and shoot ratio [19].

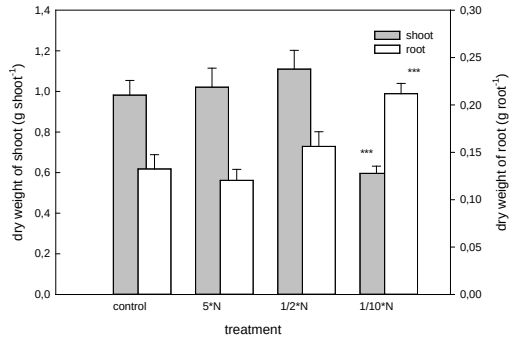

Figure 1. Changes of shoot and root dry weight (g) by nitrogen treatment: 5 times more (5*N), half amount (1/2*N) and tenths (1/10*N) than the control. n=6, ±s.e. Significant differences compared to the control: ***p<0.001.

In our experiment the 50% nitrogen deprivation (1/2*N) did not cause significant reduction in shoot dry weight, rather than conversely caused higher dry weight. This treatment slightly increased the dry weight of shoot as published by Zhang *et al*. [19].

Photosynthetic pigment content plays a critical role in photosynthesis. The amount of chlorophylls has a close correlation with photosynthetic capacity of plants [22]. The chlorophyll meter (or SPAD meter) is a simple, portable diagnostic tool for nondestructive estimation of leaf chlorophyll content [23].

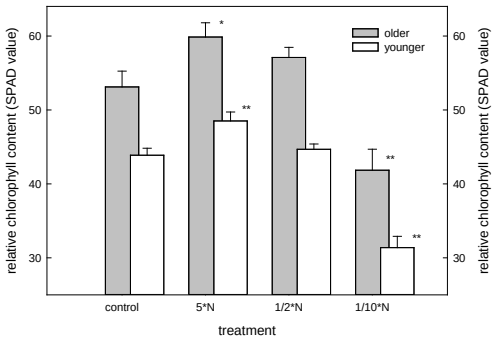

Figure 2. Changes of relative chlorophyll content (SPAD value) of older (1st) and younger (4th) leaves of cucumber seedlings affecting by nitrogen treatments: 5 times more (5*N), half amount (1/2*N) and tenths (1/10*N) than the control. n=9, ±s.e. Significant differences compared to the control: *p≤0.05, **p≤0.01, ***p≤0.001

According to our results in control plants the SPAD value was 50.8 (±8.03) in older and 43.9 (±2.3) in younger leaves of cucumber seedlings (Fig. 2). Five times more N supply resulted in higher value of SPAD by 16% in older and by 11% higher in younger leaves. Half amount of N (1/2*N) the SPAD values also were higher in older and younger leaves as well (124% and 1.8%, respectively) compared to the control value. The higher N deprivation (1/10*N) caused reduction in relative chlorophyll content. Consequently, the distribution of leaf SPAD values is affected by nitrogen availability, with differences between older and younger leaves decreasing with increasing N applications as found Yang *et al.* in rice as well [24].

Besides the amount of chlorophylls, the quality of photosynthetic pigments also determinant in plant production. In plant's chloroplasts two groups of photosynthetic pigments take place: chlorophylls and carotenoids. Chlorophylls (a and b) are sensitive for several stress which diminish the photosynthetic activity, such as nutrient deprivation, and leads to reduction in their content. Kyparissis et al., [25] and Munné-Bosch and Alegre [26] published, that this decline is a kind of down-regulation to reduce oxidative damages caused by excess light. Carotenoids play a central role in the deactivation of reactive oxygen species formation due to the thermal dissipation of excess light energy [9].

TABLE I. PHOTOSYNTHETIC PIGMENT (CHLOROPHYLL-A, B AND CAROTENOIDS) CONTENT (MG G^{-1}) IN OLDER (1ST) AND YOUNGER (4TH) LEAVES OF CUCUMBER SEEDLINGS N=3, ±S.E. P≤0.05

treatment	Photosynthetic pigment content (mg g^{-1})		
	chlorophyll-a	chlorophyll-a	carotenoids
	older leaves (1st)		
control	12.1±0.8	4.5±0.4	7.5±0.5
5*N	12.8±0.4	5.1±0.2	8.6±0.2
½*N	12.8±	5.1±0.5	7.9±0.4
1/10*N	8.8±0.5*	3.2±0.3	5.9±0.3
	younger leaves (4th)		
control	8.6±1.5	6.1±1.2	6.7±0.5
5*N	14.1±0.1*	5.9±0.2	8.6±0.3
½*N	13.5±0.3*	5.2±0.3	8.5±0.1
1/10*N	7.9±0.3	2.5±0.2*	5.5±0.2

With half amount reduced N application did not cause a decline in chlorophyll-a content and resulted in slight increase in chlorophyll-b and carotenoid contents in older leaves (Table I). Interestingly the chlorophyll-a and carotenoid contents increased in younger leaves in case of 50% N application.

Chlorophyll a fluorescence is a rapid and non-intrusive tool used to screen varieties for PSII under different stresses [13]. Photosystem II is considered to play an important role in the response of higher plants to environmental stress [27]. The reduction of CO_2 assimilation by N stress should therefore be reflected in the PSII behavior. In a fast phase of chlorophyll-fluorescence induction curve the differences between basic fluorescence (Fo) and maximal fluorescence (Fm) is a variable fluorescence (Fv). The ratio of Fv/Fm gives information about a maximal/potential efficiency of PSII [13].

The values of Fv/Fm in dicot plants under optimal conditions is 0.832±0.004 [28]. As our figure shows (Fig. 3) the control values were around this number, and five times more and half amount nitrogen supply did not caused significant differences compared to the control values. Strong nitrogen deprivation (1/10N) significantly reduced the maximal efficiency of PSII. Chlorophyll

fluorescence method is good tool for establishing plant conditions [29], but in our cases two applied nitrogen treatments (5*N and 1/2N) did not decline significantly the Fv/Fm values. The reason one: the Fv/Fm is not sensitive for the applied treatment, or reason two: in this cucumber genotype the tolerance of PSII under these nutrient supplies is adequate.

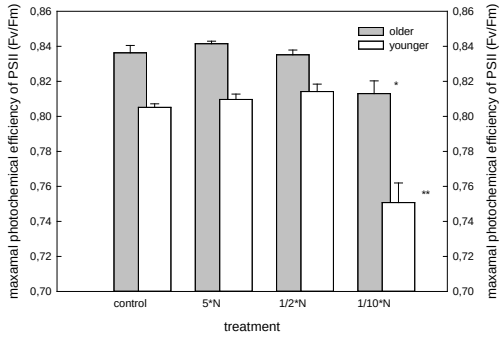

Figure 3. Values of maximal photochemical efficiency of PSII (Fv/Fm) under different nitrogen treatment: 5 times more (5*N), half amount (1/2*N) and tenths (1/10*N) than the control. n=6, ±s.e. Significant differences compared to the control: *p≤0.05, **p≤0.01.

Using adequate nitrogen (N) supply is one of the main goals of sustainable horticulture. In our experiment we made an attempt to fix the nitrogen tolerance of cucumber (*Cucumis sativus* L cv. Rajnai fürtös) in early growth stage.

In conclusion, the measured parameters connected to photosynthetic capacity of cucumber seedlings and contributed to sustainable food production. Based on our results we showed, the relatively large nitrogen deprivation did not cause significant differences compared to the optimal nitrogen supply. Younger leaves had similar reaction to the applied nitrogen supplies, it seems, that the nitrogen remobilization strategy of this cultivar is considerable. It is strongly recommended to consider N supply in different genotypes.

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