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Effect of β-alanine hydrazide derivatives on *Phaseolus vulgaris* photochemistry and CO_2 assimilation

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The effect of synthetic growth regulators β-alanine hydrazide derivatives on kidney bean (*Phaseolus vulgaris* L.) photosynthesis was investigated employing CO_2 assimilation and chlorophyll fluorescence analysis. Foliar application of the experimental growth regulator β-alanine hydrazide derivative st-120 on kidney beans significantly increased the photosynthesis rate and quantum efficiency of CO_2 assimilation. After decoding slow chlorophyll fluorescence induction kinetics, we determined a pronounced and statistically significant effect of st-120 on kidney bean maximal quantum yield of PSII (photosystem II) photochemistry and an effective quantum yield of photochemical energy conversion in PSII, associated with an increased number of oxidized PSII reaction centres. The β-alanine hydrazide derivative st-119 also enhanced the above-mentioned parameters, but the effect was not statistically significant.

Key words: β-alanine hydrazide derivatives, stilit-120, stilit-119, photosynthetic rate, slow chlorophyll fluorescence induction kinetics, kidney beans

INTRODUCTION

According to scientific literature, naturally synthesized phytohormones as well as synthetic plant growth regulators are responsible for regulation of many aspects of plant growth and development. The growth-regulating action often occurs as a synthesis of transcription factors that in turn promote synthesis of enzymes that facilitate chemical reactions in cells. Activity of phytohormones also appears as a signal transduction in cells via the chemical or physical mode that generates specific biochemical or molecular response in cells [1, 2].

At present, little is known about the impact of growth regulators β-alanine hydrazide derivatives on certain physiological processes in plants and the mode of their action. A few reports have indicated that heterocyclic amino acid derivatives (β-(6-bromopyridin-3-yl)alanine) interfere with the morphological development of *Arabidopsis* [3]. Several authors reported that N-substituted amino acids (N-phenyl- and N-(4-methylphenyl)-N-carboxiethyl-β-alanine dihydrazide) stimulated barley germination and shoot mass [4].

The aim of this research was to investigate the effect of β-alanine hydrazide derivatives on kidney bean photosynthesis. We applied slow chlorophyll fluorescence induction kinetics to determine the influence of the above-mentioned growth regulators on PSII photochemistry. To estimate the impact of β-alanine hydrazide derivatives on kidney bean CO_2 assimilation, we employed the infrared gas analysis method and quantified photosynthesis rate, and quantum efficiency of CO_2 assimilation.

MATERIALS AND METHODS

Object. Kidney bean (*Phaseolus vulgaris*) plants were grown in a soil and fine perlite mixture at a 16 h photoperiod, 25 ± 1 °C air temperature and 58 ± 2% relative air humidity. Ambient CO_2 concentration was 370 μM M^{-1} and photosynthetic active radiation (PAR) 400 μM m^{-2} s^{-1}. The growth regulators β-alanine hydrazide derivatives synthesized at Kaunas University of Technology – stilit-119 (further st-119) at a concentration of 0.25 mg/l and stilit-120 (further st-120) at a concentration of 0.05 mg/l – were sprayed on 14-day-old kidney bean leaves, whereas control plants were treated with distilled water.

Gas exchange measurements. Leaf gas exchange measurements were carried out with an infrared gas analyzer (LI-6400 Li-Cor Inc., Lincoln, NE USA). The effect of growth regulators – β-alanine hydrazide derivatives st-120 and st-119 on kidney bean CO_2 assimilation was estimated by determining the rate of photosynthesis A (μM (CO_2) m^{-2} s^{-1}) and analysing the light response curve (A vs. PAR) in which the initial slope was considered as an estimate of light use efficiency or the quantum efficiency of CO_2 assimilation (Φ_{CO_2}). Initial slopes were calculated by fitting the first three points of the light response curve to the linear regression [5, 6].

Chlorophyll fluorescence measurements. The influence of β-alanine hydrazide derivatives on kidney bean photochemistry was estimated by interpreting the slow chlorophyll fluorescence (further ChlF) induction kinetic curve (FIK) employing a PAM 2000 fluorometer (Walz, Germany) and determining the ChlF parameters: minimal fluorescence Fo and maximal fluorescence Fm in a dark-adapted sample, steady state Fs, maximal fluorescence Fm' and minimal fluorescence Fo' of a light-adapted sample. Light intensity in ChlF measurements was as follows: measuring light PAR < 0.1 μM $m^{-2} s^{-1}$, 600 Hz frequency, actinic light PAR 200 μM $m^{-2} s^{-1}$, 20 kHz frequency and light saturating flash – PAR 3500 μM $m^{-2} s^{-1}$. Infrared light was applied for fast plastoquinone pool reoxidation. Fluorescence parameters were calculated from each FIK curve. The maximal quantum yield of PSII photochemistry (Φ_{Po}) was determined as Fv / Fm where Fv equals to Fm–Fo. After actinic light application, the effective quantum yield of photochemical energy conversion in PSII (Φ_2) was calculated as $\Phi_2 = \Delta F / Fm'$. In this equation, $\Delta F = Fm' - Ft$. Photochemical quenching coefficient was calculated from the equation $qP = (Fm' - Fs) / (Fm' - Fo')$ [7–10].

Statistics. The results were statistically analysed using one-way ANOVA (analysis of variance) followed by the post hoc Bonferroni test (STATISTICA 6, StatSoft, Inc.). Fitting light response curve as well as quantifying the quantum efficiency of CO_2 assimilation, linear and logarithmic regression analysis was applied.

RESULTS

Light response. All experimental variants demonstrated typical light response curves: steeply increased A until maximal values (light-limited part) and then reached a plateau (CO_2-limited part). At the stage of low-light phase, the rate of CO_2 assimilation was limited by the electron transport rate and its capacity to regenerate ribulose 1,5-bisphosphate (RuBP) in the Calvin Cycle [11]. Following the light response curve, the st-120 growth regulator was found to influence A more than did st-119. The effect of st-120 was evident within the range of PAR from 200 μM $m^{-2} s^{-1}$ to 600 μM $m^{-2} s^{-1}$. Thereby, st-120 significantly increased A ($p < 0.01$ Bonferroni test), whereas the effect of st-119 was not significant (Fig. 1). At 800 μM $m^{-2} s^{-1}$ PAR intensity, almost all experimental variants reached the level of maximal photosynthetic rate. Furthermore, at 200 μM $m^{-2} s^{-1}$ the PAR growth regulator st-120 enhanced the rate of photosynthesis by 37.7% and 24.8% as compared to control and st-119, respectively.

In the literature, the quantum efficiency of CO_2 assimilation (Φ_{CO_2}) is described as the maximum efficiency with which a leaf can use an absorbed photon for CO_2 assimilation [12]. According to our results, Φ_{CO_2} at the PAR interval of 0–100 μM $m^{-2} s^{-1}$ was highest in the plants treated with st-120 (Fig. 2). It is worth noting that st-120 as compared to control plants considerably increased the quantum efficiency of CO_2 assimilation by 43.5% – to 0.056 μM CO_2 μM photons^{-1} ($p < 0.01$ Bonferroni test). However, kidney beans exposed to st-119 enhanced the quantum efficiency of CO_2 assimilation by 21.3% – to 0.047 μM CO_2 μM photons^{-1}.

Chlorophyll fluorescence. According to our observations, all experimental variants maintained a sufficiently high maximum quantum yield of PSII photochemistry (Φ_{Po}) which is calculated as Fv / Fm [8]. In literature, Φ_{Po} is clarified as the efficiency of PSII photochemistry in the dark-adapted state with fully oxidized PSII reaction centres [13]. Although control plants and those treated with st-119 showed a small tendency to decrease Φ_{Po} (0.80 and 0.79, respectively) compared to st-120 variant (0.82), this difference was not important. According to fluorescence theory, the maximal level of Φ_{Po} equals to 0.832 [8].

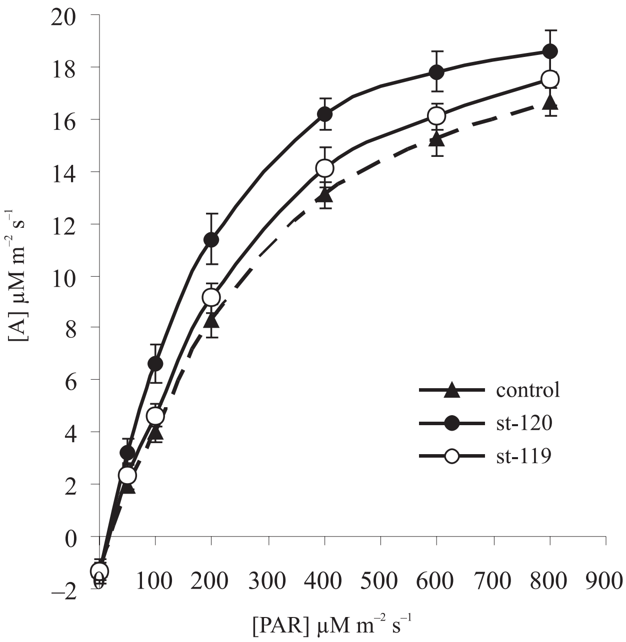

Fig. 1. Light response curve in kidney beans after application of β-alanine hydrazide derivatives st-120 and st-119. A indicates photosynthetic rate (μM $m^{-2} s^{-1}$), and PAR is photosynthetic active radiation (μM $m^{-2} s^{-1}$). Bars represent 95% confidence interval, $p < 0.05$

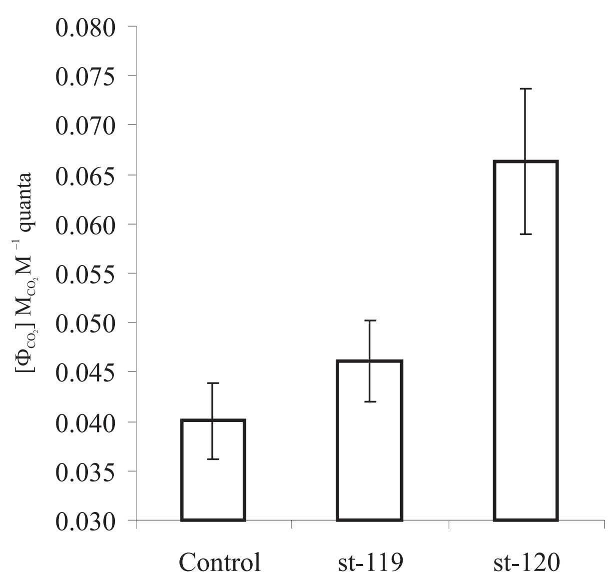

Fig. 2. Quantum efficiency of CO_2 assimilation (Φ_{CO_2}) (M CO_2 M^{-1} quanta) in kidney beans treated with β-alanine hydrazide derivatives st-120 and st-119. Bars represent 95% confidence interval, $p < 0.05$

Results of our study indicated that the values of an effective quantum yield of photochemical energy conversion in PSII (Φ_2) were enhanced in kidney beans treated with the growth regulator st-120 – Φ_2 was 14.9% higher than in control plants ($p < 0.05$, Bonferroni test). The difference of Φ_2 between st-119 and control kidney beans amounted to 3.7%.

Photochemical quenching of variable fluorescence qP, which represents the capacity of PSII reaction centres and the proportion of oxidized Q_A (quinine) to reduced one [10, 14], was highest in the st-120 variant ($p < 0.05$, Bonferroni test). The growth regulator st-119 also induced an increase in the qP value as compared to control plants, but this effect was lower than in the st-120 variant.

DISCUSSION

Our results demonstrated that foliar application of the β-alanine hydrazide derivative st-120 significantly enhanced the functioning of the photosynthetic apparatus in kidney bean. As was described by Maxwell and Johnson [7], Φ_2 measures the proportion of the light absorbed by chlorophyll associated with PSII which is used in photochemistry, and on that ground represents the rate of linear electron transport and could be an indication of overall photosynthesis [7]. Moreover, the numerical values of ChlF parameters, derived from slow ChlF induction kinetics, describe the interaction of plants with their environment and the processes taking place in plants under different external and intrinsic conditions [9]. Our observations indicate that application of the β-alanine hydrazide derivative st-120 to kidney bean plants enhances the effective quantum yield of photochemical energy conversion in PSII (Φ_2). Thus, the increase in Φ_2 was associated with higher values of qP which represents the fraction of the photochemically active (open) state of PSII reaction centres [9]. Bilger and Bjorkman [15] reported that complement of qP to 1 approximates the reduction state of Q_A – the degree of PSII reaction centre closure in the light-adapted state. Kidney bean leaves exposed to st-120 demonstrated a lower (by 35.7%) degree of PSII reaction centre closure, whereas st-119 induced a 21.4% decline as compared to control plants. Results of our study provide evidence that the higher number of oxidized PSII reaction centres in the st-120 variant markedly increased Φ_2 and gave more products such as ATP and NADPH which feed CO_2 assimilation reactions. A close relationship between Φ_{CO_2} and Φ_2 was found by Cheng et al. [16]. These authors also reported a curvilinear interrelation between qP and Φ_{CO_2}. In response to the rising PAR, the decline in qP contributed to the lowering of the quantum efficiency of CO_2 assimilation. Results of the experiment with β-alanine hydrazide derivatives showed the same tendency. Kidney beans after st-120 application showed a higher photochemical quenching as well as an increased quantum efficiency of PSII which was associated with a pronounced quantum efficiency of CO_2 assimilation (Fig. 2).

CONCLUSIONS

The application of the growth regulators β-alanine hydrazide derivatives induced kidney bean photosynthetic processes and was statistically highly significant. Infrared gas quantification and ChlF analysis showed that the growth regulator st-120, after foliar application to kidney bean, significantly increased the rate of photosynthesis, the qantum efficiency of CO_2 assimilation, an effective quantum yield of photochemical energy conversion in PSII and photochemical quenching. Growth regulator st-119 slightly but not significantly enhanced above mentioned parameters.

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β-ALANINO HIDRAZIDŲ DARINIŲ POVEIKIS PUPELIŲ FOTOCHEMINĖMS REAKCIJOMS IR CO_2 ASIMILIAVIMUI

Santrauka

Tyrimais buvo nustatytas augimo reguliatorių – β-alanino hidrazidų darinių st-120 ir st-119 – poveikis pupelių fotosintezei įvertinant CO_2 asimiliavimo ir fotocheminių reakcijų našumą. Augimo reguliatorius st-120 patikimai didino pupelių fotosintezės intensyvumą ir CO_2 asimiliavimo kvantų našumą. Įvertinę chlorofilo fluorescencijos indukcijos kinetiką, nustatėme teigiamą st-120 poveikį pupelių maksimaliam II fotosistemos fotocheminių reakcijų kvantų našumui ir efektyviam fotocheminės energijos virsmo kvantų našumui II fotosistemoje, kuris buvo tiesiogiai susijęs su oksiduotų II fotosistemos reakcinių centrų kiekiu (didesnės fotocheminio fluorescencijos slopinimo reikšmės). Tiriamasis *β*-alanino hidrazidas st-119 neturėjo statistiškai patikimo poveikio išvardytiems fotosintezės parametrams.