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Effect of Jasmonic Acid on the Stomatal and Nonstomatal Limitation of Leaf Photosynthesis in Barley Leaves

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Abstract. The effect of long-term (7 days) and short-term (up to 2 h) treatment of barley plants with jasmonic acid (JA) on the components contributing to stomatal and nonstomatal limitation of photosynthesis was studied. Net CO_2 assimilation rate (A) responses to intercellular CO_2 concentration (C_i), i.e., A/C_i curves, were used to assess the photosynthetic ability. Long-term treatment of barley plants with JA led to a noticeable decrease in both the initial slope of the A/C_i curves and the maximum A at saturating C_i. The proportion of stomatal and nonstomatal factors in limitation of photosynthesis depended on the applied JA concentration. Short-term treatment with JA affected neither the stomatal conductivity for CO_2 nor the rate of photosynthetic CO_2 assimilation. We suggest that JA may affect photosynthesis indirectly, either as a stress-modulating substance, or through the alterations in gene expression.

Key Words. Jasmonic acid—Photosynthesis—Ribulose-1,5-biphosphate carboxylase

Jasmonic acid and its methyl ester (JA-ME) received attention after the description of their physiological effects. It has been reported that they possess various biological activities, related to the growth inhibition (Yamane et al. 1980), pollen (Yamane et al. 1982) and seed (Wilen et al. 1991) germination, and promotion of leaf senescence (Ueda and Kato 1980, Satler and Thimann 1981, Weidhase et al. 1987). Evidence is available for the physiological role of jasmonates in tuberization of potato (van den Berg and Ewing 1991) and yam (Koda and Kikuta 1991) plants.

These potencies together with a wide distribution of the cyclopentanone compounds in the plant kingdom (see Meyer et al. 1984 for references, and Parthier 1991 and Sembdner and Parthier 1993 for reviews) raise the question whether jasmonates can be considered a new type of endogenous plant growth regulator (Weidhase et al. 1987).

Despite intensive research, the influence of jasmonates on the photosynthetic apparatus still remains unclear. Jasmonate-induced stimulation of stomatal closure has been suggested as an important factor in the senescence-accelerating activity of these compounds (Satler and Thimann 1981, Raghavendra and Reddy 1987). However, according to Horton (1991), JA-ME has been effective in stomatal closure only at high concentrations, when a closure could be ascribed to an irreversible toxic effect of the JA-related compounds.

Not all changes observed in the photosynthetic reactions can be attributed to the stomatal effect of jasmonates. It has been shown that plants treated with JA reveal changes in a number of photosynthetic parameters, such as a decrease in the photosynthetic rate and the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC), an increase in the rate of dark respiration and photorespiration and in the stomatal resistance to CO_2 diffusion (Popova et al. 1988). A stop in the biosynthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Weidhase et al. 1987a, Popova and Vaklinova 1988), an inhibition of the Hill reaction activity, and some changes in the kinetic characteristics of the flash-induced O_2 evolution (Maslenkova et al. 1990) have been reported to occur as a result of JA and JA-ME treatments. Considerable alterations in the chlorophyll fluorescence parameters (77 K fluorescence, chlorophyll fluorescence under F_0 and F_m) were reported for chloroplast membranes isolated from barley plants growing in the presence of increasing concentrations of JA (Ivanov and Kicheva 1993).

This paper attempts to distinguish the stomatal from the nonstomatal effects of jasmonates on the photosynthetic apparatus in barley leaves.

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Table 1. Leaf gas-exchange characteristics of barley plants after treatment with jasmonic acid for 7 days. Values are mean of six replications.[a]

Variants	Photosynthesis[b] (μmol m^{-2} s^{-1})	r'_s (s cm^{-1})	C_i (μmol mol^{-1})	r_m (s cm^{-1})	Stomatal limitation (%)
Control	7.2 ± 1.8	8.8 ± 0.2	190.9 ± 27.4	7.8 ± 1.8	30
JA 10^{-6} M	4.0 ± 0.9	15.4 ± 0.2	194.9 ± 36.8	13.4 ± 2.9	36
JA 10^{-5} M	2.1 ± 0.5	15.8 ± 0.2	265.9 ± 21.5	36.8 ± 4.5	17
$LSD_{0.05}$	1.3	0.36	43	4.2	–

[a] r'_s, stomatal resistance; C_i, intercellular CO_2 concentration; r_m, mesophyl resistance.
[b] Photosynthesis, r'_s and C_i are determined in leaves of 7-day-old plants under saturating light (500 μmol m^{-2} s^{-1}), 345 μmol mol^{-1} ambient CO_2 concentration.

Materials and Methods

Plant Material

Seeds of barley (*Hordeum vulgare* L. var. Alfa) were germinated for 2 days in two layers of moist filter paper in moist vermiculite at 25°C in the dark. Then they were transferred into petri dishes containing 40 mL distilled water or equal amounts of water solution from required JA concentrations (1 μM and 10 μM). The solutions were changed every 24 h. During the experimental period, the seedlings grew in a growth chamber under white fluorescent lamps (160 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)), with 12-h light and dark periods. Relative humidity was about 50%.

Kinetic Assay

The kinetic parameters of Rubisco K_m^c (Michaelis' constant for CO_2) and V_{max} (maximal rate of carboxylation) were estimated according to Rinehart et al. (1983) under anaerobic conditions using eight concentrations of $H^{14}CO_3^-$ (in duplicate). K_m^c and turnover numbers of Rubisco were estimated by nonlinear regression analysis. The oxygenase assay was performed as described by Lorimer et al. (1977). Rubisco protein quantity was determined by immunoenzyme assay (Metodiev and Demirevska-Kepova 1992).

Gas-Exchange Experiments

Gas-exchange measurements were performed by a portable photosynthesis system LI 6000 (Li-Cor, Lincoln, NE). Experiments were done on 7-day-old leaves at 27°C and either 500 μmol m^{-2} s^{-1} PPFD, which has been assumed to saturate the photosynthesis (for long-term effects), or 160 μmol m^{-2} s^{-1} PPFD (for evaluation of short-term effects). The photosynthetic rates and internal CO_2 concentrations were calculated according to von Caemmerer and Farquhar (1981). The response curves of photosynthesis versus C_i were obtained as described by Davis et al. (1987). The short-term effects were studied using detached leaves of 7-day-old plants. Leaves were cut under water and transferred to solutions of JA (10^{-5} M) and abscisic acid (ABA) (10^{-5} M). Control leaves were transferred to distilled water. All leaves were watered with the same solutions via their cut ends during the gas-exchange measurements. The measurements were performed every 10 min after detachment and lasted 2 h. Additional experiments were conducted after a 24-h incubation of detached leaves in JA and distilled water. Five randomly collected leaves were placed in the gas-exchange cuvette so that the leaf area was almost 10 cm^2. Each individual experiment included a determination of photoassimilation of CO_2, transpiration, and stomatal resistance at an ambient CO_2 concentration. Additionally, the maximal photosynthesis was determined at saturating CO_2 concentrations (800–1000 μmol mol^{-1}). These were obtained by breathing in the inlet of the system. Another set of experiments was conducted under the same conditions, but the plants were acclimated to higher light intensity (600 μmol m^{-2} s^{-1} PPFD) for 2 h, and all experiments were conducted under this light intensity.

Results

Long-Term Experiments

Stomatal Limitation. We used the method developed by Farquhar and Sharkey (1982) to assess the stomatal limitation. By their definition, the value of stomatal limitation is presented by the relative rise of photosynthesis, given C_i has become equal to the ambient CO_2 concentration. This value was estimated from the CO_2 responses of leaf photosynthesis (Table 1). Stomatal resistance (r'_s) to CO_2 diffusion of treated plants changed substantially with respect to control plants. The calculated values of the stomatal limitation of photosynthesis did not change when JA concentration was 10^{-6} M and substantially decreased at 10^{-5} M concentration of JA.

Nonstomatal Limitation. The results showed that a higher concentration of JA even lowered the degree of stomatal limitation of photosynthesis, although the value of stomatal resistance to CO_2 was very high. In the following analysis we try to explain these results by analyzing the CO_2 saturation kinetics of photosynthesis of control and treated plants. Figure 1 presents CO_2 curves of control and treated plants.

We used the model of Farquhar et al. (1980) and Farquhar and von Caemmerer (1982) to obtain a quantitative assessment of these effects. This model provides a mechanistic interpretation of gas-exchange data and, therefore, allows us to distinguish the effect of JA treat-

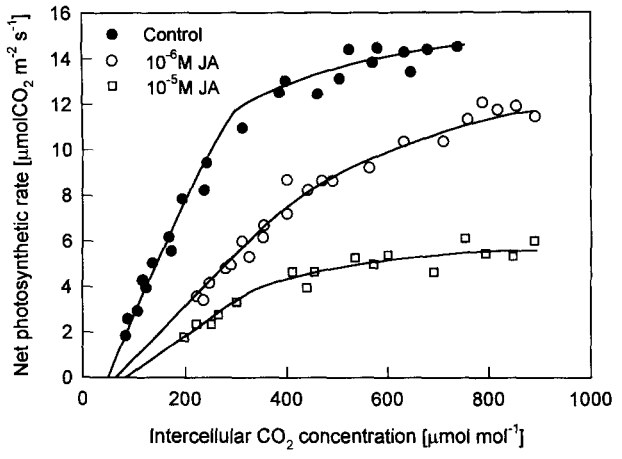

Fig. 1. Representative A/C_i curves for the control and long-term JA-treated plants. Measurements were performed under 500 μmol m^{-2} s^{-1} light intensity at 27°C using LI 6000. The curves were derived from four independent experiments. The individual points were divided into groups depending on the C_i values, and the mean values of each group (these are shown on the figure) were used to fit the model equations (Equations 1–3).

ment on some components of the system. According to the model, photosynthesis (A) is determined by the lower of two variables W_e (rate of regeneration of RuBP) and W_c (rate of carboxylation), and the intensity of "dark" respiration:

$$A = \left(1 - \frac{D}{C_i}\right) \cdot \min\{W_e, W_c\} - R_d \tag{1}$$

$$W_e = \frac{J_{\max}}{4.5 + 10.5\dfrac{D}{C_i}} \tag{2}$$

$$W_c = \frac{V_{\max} C_i}{C_i + K_m^c\left(1 + \dfrac{O_2}{K_m^o}\right)} \cdot \frac{C_i}{C_i + KA}. \tag{3}$$

We used a more simple definition of W_e because, in the experiments, the intensity of light was saturating so the intensity of electron transport could be considered to equal $J_{\max}$.

The values of Michaelis constants K_m^c (for CO_2) and K_m^o (for O_2) were obtained in vitro. These were 8.0 μM for the first and 400 μM for the second parameter. The value of activation constant for Rubisco (KA) was assumed to be 1.2 μM according to Day and Parkinson (1982).

Four parameters were estimated by fitting the model to the experimental data: $V_{\max}$, the maximal rate of carboxylation; $J_{\max}$, the maximal capacity of the electron transport; R_d, the rate of "dark" respiration; and D, a parameter corresponding to the specificity factor of Rubisco (Table 2). $V_{\max}$ decreased almost 50% upon treatment with 10^{-6} M JA, whereas $J_{\max}$ decreased by approximately 30%. The higher concentration of JA caused a more substantial decrease of $J_{\max}$; by almost 70%. Two other parameters, D and R_d, were slightly increased upon JA treatment.

Short-Term Experiments

Short-term experiments showed marked differences in the effects of JA and ABA: ABA was used as a standard for strong stomatal limitation, and was compared with that of JA. Both effectors were applied in equimolar concentrations. ABA caused a very fast decline in stomatal conductivity and transpiration in the first 5–10 min. Surprisingly, JA seemed to maintain these parameters near or even higher than those of control plants during the entire 2-h period and caused changes as long as the leaves were subjected to its action for 24 h. As shown in Figures 2 and 3, ABA caused a dynamic, almost exponential decrease in transpiration and stomatal conductivity with a half time of approximately 4 min. In JA-treated and control leaves, the parameters remained largely constant, although some oscillations occurred in their values. ABA-treated leaves achieved a new steady state within the first 30–60 min of treatment. At this state, the photoassimilation of CO_2 was 6–7 times less than that of control and JA-treated plants, in which the photoassimilation of CO_2 remained constant for the first 2 h. These results were also found in higher light acclimated plants (data not shown). The effects on the photosynthesis under high CO_2 concentration are shown in Figure 4. No significant differences were observed under these conditions.

Discussion

Inhibition of photosynthesis by long-term JA treatment might be caused by either a decrease in CO_2 conductance, mediated by stomatal closure, or by an effect on the photosynthetic electron transport and carboxylation capacity. The results presented here suggest that, upon continuous (for 7 days) treatment with 10^{-6} M JA, the capacity of carboxylation and electron transport decreases proportionally to the increase of stomatal resistance; thus, the ratio stomatal/nonstomatal limitation of photosynthesis remains largely unchanged. The higher concentration of JA decreases the RuBP carboxylation and RuBP regeneration capacities, which decreases the relative stomatal limitation and increases the nonstomatal limitation.

In interpreting the saturation curves, we conclude that

Table 2. Photosynthesis parameters for best fit of the model of Farquhar et al. (1980, 1981) to measurements of net photosynthesis of barley leaves.[a]

Variants	V_{max} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})	D (mmol m^{-3})
Control	40.4 ± 4.6	90.5 ± 7.8	1.2 ± 0.4	1.1 ± 0.13
JA 10^{-6} M	25.8 ± 1.8	69.7 ± 5.6	1.5 ± 0.3	1.3 ± 0.17
JA 10^{-5} M	22.5 ± 1.6	29.9 ± 3.5	1.6 ± 0.4	1.6 ± 0.23
$LSD_{0.05}$	3.0	8.0	0.61	0.26

[a] V_{max}, maximal capacity of carboxylation; J_{max}, maximal capacity of the electron transport; R_d, "dark" respiration intensity; D, parameter.

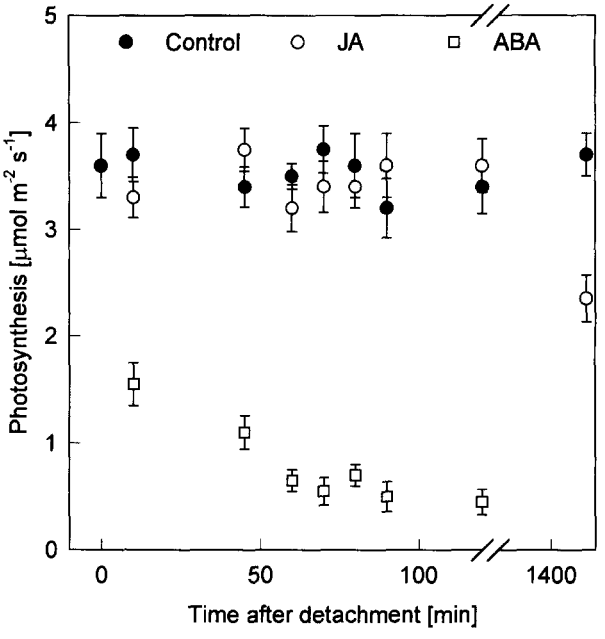

Fig. 2. Short-term effects of JA and ABA on the intensity of photoassimilation of CO_2 in detached barley leaves. Measurements were performed under 160 μmol m^{-2} s^{-1} light intensity at 27°C using LI 6000. Each point is the mean of four replications. Vertical bars represent the standard deviations.

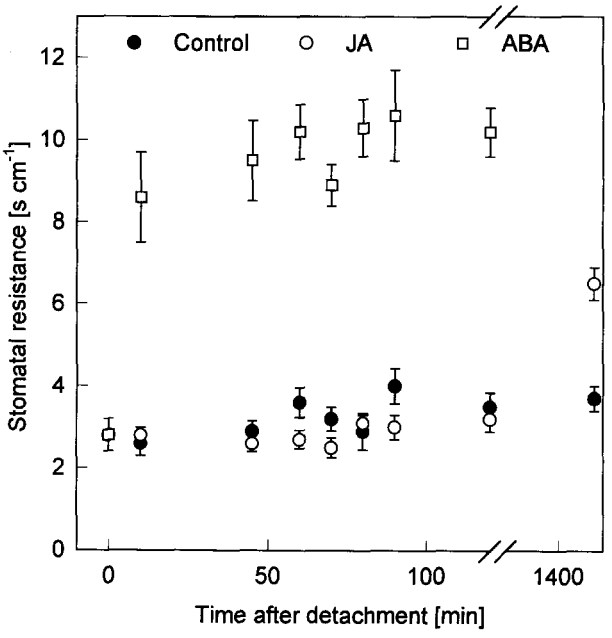

Fig. 3. Short-term effects of JA and ABA on the stomatal resistance for diffusion of CO_2 in detached barley leaves. Measurements were performed under 160 μmol m^{-2} s^{-1} light intensity at 27°C using LI 6000. Each point is the mean of four replications. Vertical bars represent the standard deviations.

JA treatment led to a reduction in the RuBP carboxylation capacity as well as in the RuBP regeneration capacity, as indicated by the decrease in the initial slopes and in the level of saturation. This dependence is obvious after treatment with the higher JA concentration (10^{-5} M). In this case, the RuBP regeneration capacity of the photosynthetic apparatus (the level of saturation) was inhibited by JA treatment more strongly than was carboxylation capacity. The long-term treatment of barley seedlings with JA caused an inhibition in Rubisco synthesis (Popova and Vaklinova 1988), an alteration of intrachloroplast structure (Popova and Uzunova 1996), and considerable changes in the polypeptides' patterns of thylakoid membrane proteins (Maslenkova et al. 1992). Isolated chloroplasts of 7-day-treated barley seedlings showed an inhibition of O_2-flash yields (Maslenkova et al. 1990). These results allow us to hypothesize that JA affects photosynthesis apart from its effects on stomatal closing because, in isolated chloroplasts of treated plants, the stomatal effects of JA were eliminated.

On the other hand, the short-term JA treatment (from minutes to 2 h) affected neither the stomatal conductivity for CO_2 nor the photosynthetic electron transport and carboxylation capacity, as compared to control plants. These observations are in contrast to the action of ABA, which exhibited a strong negative effect on the stomatal conductance for CO_2. The JA-caused decrease in photosynthetic rate and increase in r_s upon long-term treatment are a result of adaptation instead of a direct influence on specific reaction. This conclusion is supported by evi-

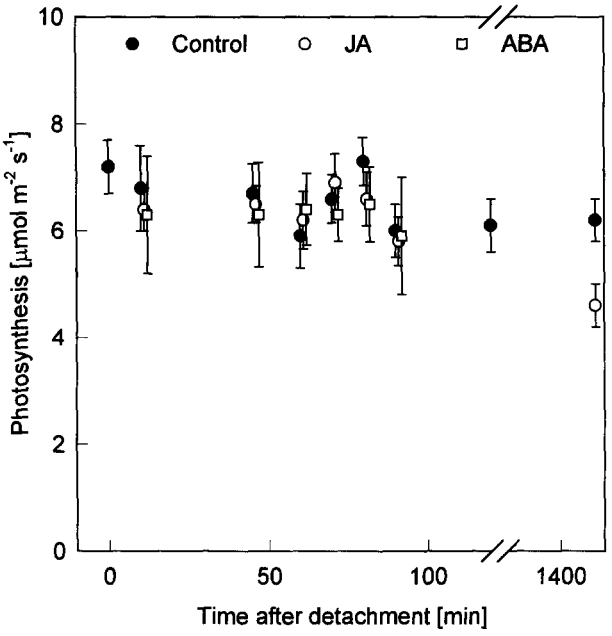

Fig. 4. Short-term effects of JA and ABA on the intensity of photoassimilation of CO_2 in detached barley leaves under high (1000 μmol mol^{-1}) CO_2 concentration. Measurements were performed under 160 μmol m^{-2} s^{-1} light intensity at 27°C using LI 6000. Each point is the mean of four replications. Vertical bars represent the standard deviations.

dence which confirms the role of JA as a stress-modulating compound as well as by the available information on the action of jasmonates on gene expression.

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