Response of photosynthesis of *Pisum sativum* to salt stress as affected by methyl jasmonate

M. VELITCHKOVA* and I. FEDINA**

*Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. bl. 21, BL-1113 Sofia, Bulgaria**
*Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. bl. 21, BL-1113 Sofia, Bulgaria***

Abstract

10^{-5} M methyl jasmonate (JA-Me) treatment itself did not considerably change the $^{14}CO_2$ fixation, parameters of room temperature chlorophyll fluorescence induction, proline content, and Na^+ as well as Cl^- accumulation. Salt stress (30 mM NaCl) lead to a decrease of both $^{14}CO_2$ fixation and relative water content, and to an increase of proline content. Immediate nonvariable fluorescence (F_0) also increased and the variable to maximal fluorescence ratio (F_v/F_m) decreased. Pretreatment with JA-Me for 3 d before salt treatment diminished the inhibitory effect of NaCl on the rate of $^{14}CO_2$ fixation, protein content, and activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase. The Na^+ and Cl^- contents in leaves decreased in JA-Me pretreated plants. The JA-Me pretreatment prevented the increase of F_0 level and restored the values of F_v/F_m.

Additional key words: chlorophyll fluorescence; Cl; Na; NaCl; pea; proline; relative water content; ribulose-1,5-bisphosphate carboxylase/oxygenase; salt stress.

Introduction

Exposure of plants to salt stress results in changes in most physiological and biochemical processes resulting in a disturbance of normal growth and development. Salt stress induces reduction in primary photochemical activities (*e.g.*, Kaiser *et al.* 1983), inhibition of CO_2 assimilation (*e.g.*, Seemann and Sharkey 1986), and

Received 27 August 1997, *accepted* 8 October 1997
*Fax: 359 2 971 2493; e-mail: mayav@obzor.bio21.acad.bg
Abbreviations: ABA, abscisic acid; Chl, chlorophyll; F_0, immediate nonvariable fluorescence; F_m, maximal fluorescence; F_v -variable fluorescence; JA-Me, methyl jasmonate; *P*, rate of $^{14}CO_2$ fixation; PS, polystyrol; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC, relative water content.
Acknowledgements: The authors thank Dr. K. Demirevska-Kepova for performing the ELISA determination of RuBPCO concentration. This research was supported by a grant from National Fund for Scientific Investigations (K-511), Bulgaria.

alteration in polypeptide profiles (*e.g.*, Hurkman *et al.* 1989). The response of plants to salt stress depends on many factors, but phytohormones are thought to be among the most important endogenous substances involved in the mechanisms of tolerance or susceptibility of various plant species. The tolerance of certain plants to different stresses is probably related to their capacity to produce a high amount of abscisic acid (ABA). ABA plays a central role in regulating osmotic adaptation of the plants, as well as in the synthesis of proline (*e.g.*, Aspinall and Paleg 1981, Stewart and Voetberg 1985). Our previous results (Fedina *et al.* 1994) also showed the protective effect of ABA against NaCl stress.

Jasmonic acid and its fragrant methyl ester are the major representatives of a group of native plant bioregulators called jasmonates. They both stimulate and inhibit different biological systems (Ueda and Kato 1980, 1981, Saniewski and Czapski 1983). Exogenously applied jasmonates are stressors causing typical stress responses (Popova and Uzunova 1996). Most evident is the synthesis of novel abundant proteins (Parthier 1990, 1991). At present it is difficult to prefer one or another view, because neither the role of jasmonate nor the primary sites of its action are known. Some observations point to cell membranes: trigger function of jasmonates becomes likewise plausible by physico-chemical or chemical interactions with biological membranes. As a hypothesis, cell membrane constituents can be released, and acting as elicitors they induce alteration in gene expression (Thomas 1986).

The concentration of endogenous jasmonates increases in response to external stimuli, such as wounding (Creelman *et al.* 1992), mechanical forces (Falkenstein *et al.* 1991), and osmotic stress (Parthier *et al.* 1992). The stress-induced rise in endogenous jasmonates suggests that they might be involved in the transduction chain between external stress impulse and internal stress response. The effects of jasmonates show similarity to ABA action not only in the promotion of leaf senescence, but also in the induction and accumulation of several abundant protein classes in barley leaf segments (Weidhase *et al.* 1987), which might be important in acquired stress tolerance.

This study aimed at evaluating the role of exogenously applied JA-Me in counteracting the salt stress. The established protective effect was discussed with respect to involvement of JA-Me in osmoregulation or osmoprotection based on increased proline accumulation and changes in concentrations of Na^+ and Cl^- in leaves.

Materials and methods

Plants: Seeds of *Pisum sativum* L. cv. Ran 1 germinated for 3 d at 25 °C. Seedlings were then transferred to pots containing distilled water. After 5 d, some of the plants were placed in 10^{-5} M solution of JA-Me, pH 5.5. After 3 d, 30 mM NaCl was added both to JA-Me treated and non-treated seedlings. Samples for analysis were taken 4 d after salinization. Control plants were kept in distilled water. During the experimental period, the seedlings grew in a growth chamber under white fluorescent lamps (160 μmol m^{-2} s^{-1}) with a 12 h photoperiod. Day/night temperatures were 25/20 °C, and relative humidity was about 50 %.

Protoplasts were isolated by the method of Huber and Edwards (1975). The enzyme medium contained 0.5 M sorbitol, 1 mM KH_2PO_4, 1 mM $MgCl_2$, 2 % cellulase (*Onozuka R-10*), and 0.5 % pectinase (*Macerozyme*) at pH 5.5. Leaf segments were incubated with the enzyme medium at 30 °C for 3 h.

Chloroplasts from green leaves of plants grown in different solutions were isolated according to Jensen and Bassham (1966). The final pellet was resuspended in a medium containing 10 mM Tricine-NaOH (pH 8.0), 0.33 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. Chlorophyll (Chl) concentration was determined by the method of Lichtenthaler (1987).

Relative water content (RWC) was measured as described by Morgan (1986) except that turgid mass was obtained after soaking the leaves for 2 h in distilled water.

$^{14}CO_2$ fixation (*P*) was measured in intact leaves or isolated chloroplasts. The leaves in a closed chamber were kept for 20 min with 7.4 MBq ^{14}C under an irradiation of 920 μmol m^{-2} s^{-1}, and temperature of 25 °C. The plant material was killed with boiling 80 % ethanol, and total radioactivity was measured. The reaction mixture for $^{14}CO_2$ fixation in chloroplasts contained 0.5 M sorbitol, 2 mM $MgCl_2$, 1 mM KH_2PO_4, 1 mM $MnCl_2$, 1 mM EDTA, 20 mM Tris-HCl, pH 7.5, and 6 mM $NaH^{14}CO_3$. $^{14}CO_2$ fixation assays were performed in a total volume of 2 cm^3, at 30 °C for 20 min.

Enzyme assays: 1 g fresh mass of leaves was ground with 5 cm^3 of cold extraction medium, containing 0.33 M sorbitol, 0.05 M Hepes-NaOH, 0.002 M KNO_3, 0.002 M EDTA, 0.001 M $MnCl_2$, 0.001 M $MgCl_2$, 0.0005 M K_2HPO_4, 0.02 M NaCl, and 0.2 M sodium isoascorbate (pH 7.6). The homogenate was centrifuged at 20 000×*g* for 15 min, and the supernatant was used directly for enzyme assay.

The assay mixture for ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPC (EC 4.1.1.39) contained 50 mM Hepes-NaOH (pH 8.0), 20 μmol $MgCl_2$, 1 μmol DDT, 20 μmol $NaHCO_3$ (containing 1.48 MBq of ^{14}C, specific radioactivity 529 MBq mol^{-1}), and enzyme extract equivalent to 0.3-0.4 mg of protein. The reaction volume was 1 cm^3. Reaction at 25 °C was initiated by the addition of 2 μmol RuBP, and stopped after 1 min with 6 M HCl. The amount of $^{14}CO_2$ fixed into acid-stable products was measured in a liquid scintillation counter.

The ELISA determination of RuBPCO was a modification of the method of Demirevska-Kepova *et al.* (1990). Wells of *PS* microtiter plates (*Labor-Technik*, Greiner, Germany) were covered with standard or unknown RuBPCO solution. Plates were incubated at 4 °C for 18 h, and the wells were washed tree times with PBS that contained 0.05 % *Triton X-100*, blocked with PBS, containing 1 % ovalbumin for 1 h at 30 °C. An IgG-enriched rabbit anti-RuBPCO-immunoserum (1 : 2500) in PBS containing 0.5 % ovalbumin was added to the wells. Plates were incubated at 30 °C for 90 min and washed as above. To each well, 100 mm^3 of peroxidase conjugated goat anti-rabbit IgG diluted 1 : 1000 in PBS was added. The plates were washed after 30 min incubation at 30 °C. Finally, the colour was developed by adding 100 mm^3 freshly prepared solution that contained 2 g m^{-3} *o*-phenylenediamine dihydrochloride and 0.015 % H_2O_2 in phosphate-citrate buffer, pH 5.0 (for peroxidase reaction).

After 5 min incubation at room temperature, the reaction was stopped with 50 mm^3 2 M H_2SO_4, and absorbance was measured at 492 nm. Parallel controls were performed without peroxidase conjugated immunoserum, without antigene, and with pre-immunoserum. The absorbance of each well was measured using an ELISA reader.

Chl fluorescence induction of leaf disks was measured using a modulation Chl fluorometer *PAM 101* (*H. Walz*, Effelrich, Germany). The measuring beam was set at 1.6 kHz. The leaf disks was dark adapted for at least 5 min before measuring.

Low temperature (77 K) fluorescence spectra of isolated thylakoids were registered using a *Jobin Yvon JY3* spectrofluorometer equipped with a liquid nitrogen device and a red sensitive photomultiplier (*Hamamatsu*, Japan). The data were digitized, collected by an built-in A/D converter, and transferred to an on-line *IBM*-compatible computer for further retrieval and analysis. Chl concentration was 10 mg m^{-3}.

Other analyses: For mineral analysis, plant material was dried at 80 °C and powdered in a mortar. Cl^- content was measured by silver ion titration using the method of Cotlove (1963). Na^+ was determined by flame photometry. Proline was determined by the method of Bates *et al.* (1973). Protein was determined by the method of Bradford (1976).

Results

The pea plants were very sensitive to salinity, and concentrations higher than 30 mM NaCl had harmful effect on them. The used concentration reduced seedling growth and the rate of $^{14}CO_2$ fixation (P). Pretreatment with JA-Me alleviated the inhibitory effect of NaCl, and P of these seedlings was higher in comparison with that of salt treated plants (Table 1).

Table 1. The photosynthetic rate (P) of leaves [$\mu g(CO_2)\ m^{-2}\ s^{-1}$], protein content [$g\ kg^{-1}$(f.m.)], proline content [$mmol\ kg^{-1}$(f.m.)], relative water content (RWC) [%], activity [$mmol(^{14}CO_2)\ kg^{-1}$(protein) s^{-1}] and quantity [%] of RuBPCO in leaves, contents of Na^+ and Cl^- [$g\ kg^{-1}$(d.m.], and P of protoplasts [$mmol(^{14}CO_2)\ kg^{-1}$(Chl) s^{-1}] in protoplasts of pea seedlings treated for 3 d with 10^{-5} M JA-Me before treatment with 30 mM NaCl. Values are means ± S.E. of 3 separate experiments.

Treatment	P(leaves)	Protein	Proline	RWC	P(protopl.)	RuBPCO activity	RuBPCO content	Na^+	Cl^-
Control	720±60	15.8±1.6	840±32	93.4	871±56	9.4±0.9	100.0	1.8±0.2	1.7±0.3
JA-Me	570±40	13.6±0.8	1020±41	93.1	768±68	8.3±0.3	97.3	1.6±0.1	1.8±0.2
NaCl	280±20	8.6±0.3	1480±47	88.3	356±42	6.1±0.3	71.2	28.8±1.4	39.1±3.4
JA-Me +NaCl	440±30	9.4±0.4	1210±23	91.8	591±47	7.7±0.5	81.4	19.4±2.5	26.3±2.7

The decline of photosynthesis in response to increased salinity is to some extent the result of decreased stomatal conductance. However, in several instances

decreased mesophyll conductance has also been observed which suggests inhibition of photosynthesis at the biochemical level. To eliminate the action of stomata we used isolated protoplasts to study the effect of NaCl on the photosynthetic process and involvement of JA-Me in photosynthetic responses to salinity.

The *P* of protoplasts isolated from plants subjected to salinity for 3 d was inhibited by about 60 % (Table 1). The observed inhibition was due to the direct effect of NaCl on photosynthesis because stomatal control was removed. *P* of protoplasts isolated from plants pretreated with JA-Me before salt treatment was *ca.* 66 % higher than that of protoplasts isolated from plants treated with NaCl only. Feeding whole plants through the roots with JA-Me for 3 d slightly reduced *P* of protoplasts isolated from these plants. JA-Me added exogenously (*in vitro*) to the protoplasts isolated from control plants did not affect *P* (values not shown). We never found an effect on *P* after addition of JA-Me to isolated mesophyll protoplasts, nevertheless, the phytohormone was added to the suspension just before exposure to $NaH^{14}CO_3$ or the protoplasts were pretreated for 15, 30, and 60 min.

The RuBPCO activity was almost unaffected when the plants were treated with 10^{-5} M JA-Me (Table 1). Exposure of plants to salinity lead to some decrease of enzyme activity.

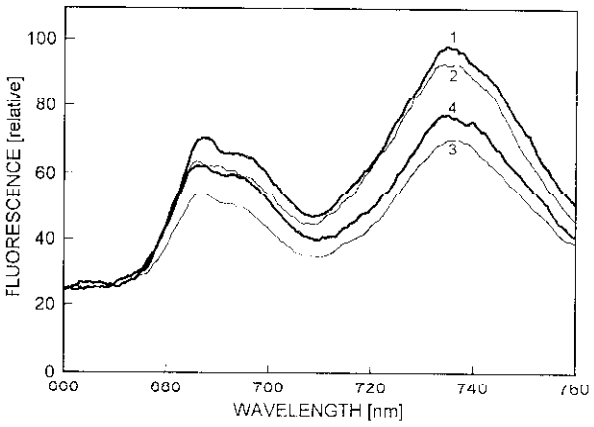

Fig. 1. 77 K fluorescence emission spectra of chloroplasts isolated from control (*1*), JA-Me (*2*), NaCl (*3*), and JA Me+NaCl (*4*) treated pea plants. The resuspending medium contained 10 mM Tricine-NaOH (pH 8.0), 330 mM saccharose, 10 mM NaCl, and 5 mM $MgCl_2$. Chlorophyll concentration was 10 g m^{-3}. Excitation wavelength was 436 nm.

The low temperature fluorescence spectra upon excitation with λ = 436 nm for control, JA-Me-, NaCl, and JA-Me+NaCl-treated chloroplasts (Fig. 1) showed that JA-Me pretreatment lead to a decrease of the fluorescence maximum in the region 685-695 nm attributed to pigments associated with PS2-LHC2 complex. The maximum at 735 nm, which is currently believed to belong to PS1-associated pigments, was not altered. The NaCl-treated chloroplasts showed a reduced fluorescence in both regions, but the decrease of intensity at 735 nm was more

pronounced. Pretreatment of plants with JA-Me before exposure to salt stress restored the fluorescence yield in the region 685-695 nm to the level obtained for JA-Me treated plants. The fluorescence intensity at 735 nm was also enhanced but it did not reach the level for control and JA-Me treated chloroplasts.

Table 2. The ratios F735/F685, F735/F695, and F685/F695 obtained from 77 K fluorescence emission spectra (Fig. 1) of chloroplasts isolated from control, JA-Me-, NaCl-, and JA-Me+NaCl-treated pea plants upon excitation at 436 and 472 nm. Mean values ± S.E. were calculated from 3 independent experiments.

Treatment	Excitation 436 nm			Excitation 472 nm		
	F735/F685	F735/F695	F685/F695	F735/F685	F735/F695	F685/F695
Control	1.34±0.03	1.44±0.03	1.08±0.02	1.19±0.03	1.33±0.02	1.12±0.02
JA-Me	1.36±0.01	1.49±0.01	1.09±0.02	1.15±0.07	1.27±0.08	1.10±0.01
NaCl	1.25±0.04	1.38±0.04	1.10±0.01	1.11±0.02	1.25±0.05	1.12±0.03
JA-Me+NaCl	1.31±0.04	1.40±0.07	1.07±0.04	1.10±0.05	1.25±0.06	1.18±0.04

The ratio F735/F685 is usually used as a measure for energy distribution between both photosystems. The JA-Me treatment did not alter the F735/F685, but after NaCl treatment, F735/F685 decreased: it was restored near to the level in the control when plants were pretreated with JA-Me before salinization (Table 2). Hence NaCl may affect the energy transfer between both photosystems decreasing the extent of spillover. After the JA-Me pretreatment the ratios F735/F685 reached the control values thus indicating a similar energy distribution after salinization of JA-Me treated plants.

Table 3. Room temperature chlorophyll (Chl) *a* fluorescence induction in leaf disk from control, JA-Me-, NaCl-, and JA-Me+NaCl-treated plants. Parameters F_0 and F_v/F_m represent immediate nonvariable fluorescence and the ratio of variable fluorescence to maximal fluorescence, respectively.

Treatment	F_0	F_v/F_m
Control	0.088	0.728
JA-Me	0.087	0.732
NaCl	0.106	0.674
JA-Me+NaCl	0.082	0.714

The JA-Me treatment did not cause any remarkable changes of F_0 and F_v/F_m (Table 3). Salinity stress resulted in an about 10 % increase of F_0. Pretreatment with JA-Me decreased the F_0 level to that of control chloroplasts. These results agree with those on the preventing effect of JA-Me pretreatment against salt-induced changes of the low temperature fluorescence in the region of 685-695 nm. Exposure to high concentration of NaCl decreases F_v/F_m. JA-Me pretreatment restores the F_v/F_m value thus indicating that JA-Me protects the photosynthetic apparatus against salt stress.

For better understanding of the established protective effect of JA-Me we investigated the proline content and Na^+ and Cl^- accumulation in plant tissues. Proline accumulation as a response to salt stress is known in many plant species (Antolín and Sanchez-Díaz 1992). Proline content in JA-Me treated seedlings increased in parallel to the proline content in seedlings subjected to salt stress (Table 1), but no synergistic effect was found when NaCl was supplied to JA-Me pretreated plants. The high content of free proline in NaCl treated plants might be due to an appearance of mild osmotic stress (additionally to ionic stress), because RWC decreased to 88.3 % (Table 1). This effect of NaCl was smaller in JA-Me pretreated plants. JA-Me itself did not affect the leaf water status.

Concentrations of Na^+ and Cl^- (Table 1) of salt treated seedlings increased about 25 times as compared with control. As a result of pretreatment with JA-Me, the accumulation of Na^+ and Cl^- decreased by 34 % in comparison with salt stressed plants.

Discussion

Exposure of pea seedlings to 30 mM NaCl results in inhibition of P_n RuBPCO activity, and electron transport rate in intact leaves. $^{14}CO_2$ fixation by protoplasts isolated from the plants subjected to salinity for 3 d was reduced by more than 50 %. This confirms non-stomatal limitation of photosynthesis by NaCl.

The measurements of variable fluorescence in whole leaves supported the notion that the photosynthetic apparatus of the leaves was impaired by salt treatment. The obtained increase of F_0 may reflect some damage in efficient energy interaction between antenna Chl *a* molecules and reaction centres. JA-Me pretreatment prevented the salt-induced decrease of F_v/F_m thus indicating the protective role of JA-Me for PS2 efficiency.

Possible reason for the observed inhibition of P_n and RuBPCO activity could be the inhibiting effect of salinity stress on synthesis of proteins, including the synthesis of RuBPCO (Table 1). The observed change in the activity of RuBPCO was probably in some extent due to different rates of protein synthesis, including the synthesis of RuBPC, which decreased by about 30 %. Herrmann *et al.* (1989) also show that in excised barley leaves JA-Me induces inhibition of synthesis of photosynthesis-related proteins such as the small and large subunits of RuBPCO and proteins of the light-harvesting complex. Lal *et al.* (1996) established also in barley that with the decrease in RuBPCO activity during stress there was a large corresponding decrease in RuBPCO content, while there was no significant change in the RuBPCO activation.

The hormonal control of stomatal movement is one of the impressive adaptations of plants to osmotic stress. According to Satler and Thimann (1981) the exogenous treatment of barley leaves with JA-Me causes closure of the stomata. JA-Me treatment decreased both stomatal conductance and transpiration rate of pea leaves (results not shown). The pretreatment with JA-Me before salt treatment decreased also Na^+ and Cl^- accumulation in leaves which could be one of the reasons for the observed protective action of JA-Me. Unavoidable uptake of specific ions by plants

and accumulation of these ions in leaves may result in an inhibition of photosynthesis (Robinson *et al.* 1983, Seemann and Critchley 1985). This change in the endogenous level of monovalent ions probably inhibits electron transport.

JA-Me enhances proline accumulation which may regulate osmotic balance of the cell (Stewart and Lee 1974). JA-Me may act as stress modulator by changing the stress responses of plants in a complex manner. In our case the phytohormone decreased accumulation of Na^+ and Cl^- ions in the leaves and increased the level of endogenous free proline. JA-Me also inhibits stomatal opening (Raghavendra and Reddy 1987, Satler and Thimann 1981) which results in lowering of transpiration. The decreased transpiration may probably lead to a limited transport of Na^+ and Cl^- from root to shoot. We suggest that low concentrations of Na^+ and Cl^- in the shoot are important but not sufficient to ensure plant survival at high salinity.

References

Antolín, M.C., Sánchez-Díaz, M.: Photosynthetic nutrient use efficiency, nodule activity and solute accumulation in drought stressed alfalfa plants. - Photosynthetica **27**: 595-604, 1992.

Aspinall, D., Paleg, L.G.: Proline accumulation: Physiological aspects. - In: Paleg, L.G., Aspinall, D. (ed.): Physiology and Biochemistry of Drought Resistance in Plants. Pp. 205-241. Academic Press, New York 1981.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. - Plant Soil **39**: 205-207, 1973.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Cotlove, E.: Determination of the true chloride content of biological fluids and tissues. Analysis by simple non-isotopic methods. - Anal. Chem. **35**: 101-105, 1963.

Creelman, R.A., Tierney, M.L., Mullet, J.E.: Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. - Proc. nat. Acad. Sci. USA **89**: 4938-4941, 1992.

Demirevska-Kepova, K., Kandiisky, D., Juperlieva-Mateeva, B.: Quantative determination of ribulose-1,5-bisphosphate carboxylase/oxidase in leaf extracts from barley plants by enzyme-linked immunosorbent assay. - Photosynthetica **24**: 637-640, 1990.

Falkenstein, E., Groth, B., Mithofer, A., Weiler, E.W.: Methyl jasmonate and linolenic acid are potent inducers of tendril coiling. - Planta **185**: 316-322, 1991.

Fedina, I.S., Tsonev, T.D., Guleva, E.I.: ABA as a modulator of the response of *Pisum sativum* to salt stress. - J. Plant Physiol. **143**: 245-249, 1994.

Herrmann, G., Lehmann, J., Peterson, A., Sembdner, G., Weidhase, R.A., Parthier, B.: Species and tissue specificity of jasmonate-induced abundant proteins. - J. Plant Physiol. **134**: 703-709, 1989.

Huber, S.C., Edwards, G.E.: An evaluation of some parameters required for the enzymatic isolation of cells and protoplasts with CO_2 fixation capacity from C3 and C4 grasses. - Physiol. Plant. **35**: 203-209, 1975.

Hurkman, W.J., Fornary, C.S., Tanaka, C.K.: A comparison of the effect of salt on polypeptides and translatable mRNAs in roots of a salt-tolerant and salt-sensitive cultivar of barley. - Plant Physiol. **60**: 1444-1456, 1989.

Jensen, R.G., Bassham, J.A.: Photosynthesis by isolated chloroplasts. - Proc. nat. Acad. Sci. USA **56**: 1095-1101, 1966.

Kaiser, W.M., Weber, H., Sauer, M.: Photosynthetic capacity, osmotic response and solute content of leaves and chloroplasts from *Spinacia oleracea* under salt stress. - Z. Pflanzenphysiol. **115**: 15-27, 1983.

Lal, A., Ku, M.S.B., Edwards, G.E.: Analysis of inhibition of photosynthesis due to water stress in the C_3 species *Hordeum vulgare* and *Vicia faba:* Electron transport, CO_2 fixation and carboxylation capacity. - Photosynth. Res. **49**: 57-69, 1996.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. - In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. 148. Pp. 350-382. Academic Press, San Diego - New York - Berkeley - Boston - London - Sydney - Tokyo - Toronto 1987.

Morgan, J.A.: The effects of N nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum* L.). - Plant Physiol. **80**: 52-58, 1986.

Parthier, B.: Jasmonates: hormonal regulators or stress factors in leaf senescence? - J. Plant Growth Regul. **9**: 1-7, 1990.

Parthier, B.: Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions. - Bot. Acta **104**: 446-454, 1991.

Parthier, B., Bruckner, C., Dathe, W., Hause, B., Herrmann, G., Knofel, H.D., Kramell, H.M., Lehmann, J., Miersch, O., Reinbothe, S., Sembdner, G., Wasternack, C., Nieden, U.: Jasmonates: metabolism, biological activities, and modes of action in senescence and stress responses. - In: Karssen, C.M., Van Loon, L.C., Vreugdenhil, D. (ed.): Progress in Plant Growth Regulation. Pp. 276-285. Kluwer Academic Publ., Dordrecht 1992.

Popova, L.P., Uzunova, A.N.: Changes in the chloroplast ultrastructure of barley leaves under treatment with jasmonic acid. - Photosynthetica **32**: 635-639, 1996.

Raghavendra, A.S., Reddy, K.B.: Action of proline on stomata differs from that of abscisic acid, G-substances, or methyl jasmonate. - Plant Physiol. **83**: 732-734, 1987.

Robinson, S.P., Downton, W.J.S., Millhouse, J.A.: Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. - Plant Physiol. **73**: 238-242, 1983.

Saniewski, M., Czapski, J.: The effect of methyl jasmonate on lycopene and ß-carotene accumulation in ripening red tomatoes. - Experientia **39**:1373-1374, 1983.

Satler, S.O., Thimann, K.V.: Le jasmonate de méthyle: nouveau et puissant promoteur de la sénescence des feuilles. Comp. rend. Acad. Sci. Paris, Sér. III **293**: 735-740, 1981.

Seemann, J.R., Critchley, C.: Effect of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. - Planta **164**: 151-162, 1985.

Seemann, J.R., Sharkey, T.D.: Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool sizes in *Phaseolus vulgaris* L. - Plant Physiol. **82**: 555-560, 1986.

Stewart, C.R., Lee, J.A.: The role of proline accumulation in halophytes. - Planta **120**: 279-289, 1974.

Stewart, C.R., Voetberg, G.: Relationship between stress-induced ABA and proline accumulation and ABA-induced proline accumulation in excised leaves. - Plant Physiol. **79**: 24-27, 1985.

Thomas, H.: The role of polyunsaturated fatty acids in senescence. - J. Plant Physiol. **123**: 97-105, 1986.

Ueda, J., Kato, J.: Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). - Plant Physiol. **66**: 246-249, 1980.

Ueda, J., Kato, J.: Promotive effect of methyl jasmonate on oat leaf senescence in the light. - Z. Pflanzenphysiol. **103**: 357-359, 1981.

Weidhase, R.A., Kramell, H.-M., Lehmann, J., Liebisch, H.-W., Lerbs, W., Parthier, B.: Methyljasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. - Plant Sci. **51**: 177-186, 1987.