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BRIEF COMMUNICATION

Response of *Scenedesmus incrassatulus* to salt stress as affected by methyl jasmonate

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Abstract

Exposure of the freshwater green alga *Scenedesmus incrassatulus* Bohl, strain R-83 to salt stress (175 mM NaCl) resulted in a reduction of its growth and $^{14}CO_2$ fixation and in an increase of accumulation of free proline and malondialdehyde (MDA). The accumulation of proline in the light was higher than in dark. NaCl significantly inhibited the Fe-induced release of organic chelators from the cells. Exogenously supplied 10^{-4}M methyl jasmonate (JA-Me) did not considerably change the $^{14}CO_2$ fixation, but increased proline and MDA accumulation in the cells and moderately inhibited the release of chelators from cells. JA-Me supplied simultaneously with NaCl helps the algae to counteract the salt stress.

Additional key words: ion accumulation, iron chelators, malondialdehyde, photosynthesis, proline.

Jasmonic acid (JA) and its methyl ester (JA-Me) are endogenous growth regulators identified in many plant species. They modulate expression of numerous genes and influence specific aspects of plant growth, development, and responses to biotic and abiotic stresses (Creelman and Mullet 1997). Salt stress is a complex stress provoking osmotic and ion toxic effects. Ion toxic effects could have caused membrane damage and hence triggered the release of the lipid precursors for jasmonate synthesis (Moons *et al.* 1997). Since organic chelators diminish the capability of Fe to catalyze lipid peroxidation in biomembranes the endogenous level of chelators may be important for stress tolerance.

This study aimed at evaluating the role of exogenously applied JA-Me to the green alga *Scenedesmus incrassatulus* in counteracting the salt stress and changing the stress-induced responses such as intracellular accumulation of malondialdehyde (MDA) and proline, photosynthetic rate, cell growth and the release of organic Fe-binding chelators.

The green alga *Scenedesmus incrassatulus* Bohl, R-83 was cultivated in 3.5-fold diluted inorganic nutrient medium which contained 60 μM $FeCl_3$ and did not contain EDTA (Benderliev and Ivanova 1994). Iron-sufficient cells from logarithmically growing cultures were used as inoculum. The medium also contained 175 mM NaCl or 10^{-4} M JA-Me or both NaCl and JA-Me. The suspension was kept at temperature of 30 °C in dark or under continuous irradiance 170 μmol m^{-2} s^{-1} supplied by 40 W daylight fluorescent lamps and bubbled with 100 dm^3 m^{-3} h^{-1} of 2 % CO_2 in air.

Cell-supernatants were aerated 80 min with 2 % CO_2 in air to favor the formation of stable organic complexes of iron. Then samples were taken for determination hydroxylamine-labile (HA-labile) and total Fe. HA-labile Fe was determined as follows: to 5 cm^3 cell-free supernatant was added 0.2 cm^3 solution of 5.9 M CH_3COONH_4 in 3 M CH_3COOH (pH 4.5). Then 0.1 cm^3 2.87M $NH_2ON.HCl$ 0.2 cm^3 solution of 25.25 μM *o*-phenanthroline chloride were added and absorbance of

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Abbreviations: HA - hydroxylamine; JA-Me - methyl jasmonate; MDA - malondialdehyde; TBA - thiobarbituric acid; TCA - trichloroacetic acid.

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the Fe(II)-phenanthroline complex was red after 15 min at 492 nm on a spectrophotometer *Specol* (*Carl Zeiss*, Jena, Germany). Total iron was determined by the same procedure, but instead of hydroxylamine to the 5 cm^3 cell-free supernatant 0.1 cm^3 fresh daily-prepared 10 % solution of ascorbic acid was added. HA-stable Fe, as a measure for the released chelators, was computed as the difference between total Fe and HA-labile Fe. Method of Esterbauer and Cheeseman (1990) was followed for determination of MDA. $^{14}CO_2$ fixation assays were performed in a total volume of 2 cm^3 algal suspension at 25 °C for 20 min and with 7.4 MBq $NaH^{14}CO_3$. Proline was determined according to procedure described by Schobert (1977) and after the method of Bates *et al.* (1973). Cl^- was measured by silver ion titration by the method of Cotlove (1963). Na^+ was determined by flame photometry.

The photosynthesis was inhibited from the beginning of salt stress. The $^{14}CO_2$ fixation in dark was much lower than in the light. JA-Me itself has moderate negative effect on $^{14}CO_2$ fixation. Treatment with JA-Me alleviated the inhibitory effect of NaCl both in the light and in dark incubated cells. Supplying simultaneously NaCl and JA-Me resulted in increase of $^{14}CO_2$ and dry mass of treated cells in comparison with that of NaCl treated (Table 1).

Table 1. Dry mass, photosynthetic $^{14}CO_2$ fixation, proline and MDA contents and chelators in *Scenedesmus incrassatulus* as affected by 125 mM NaCl and 10^{-4} M JA-Me. Means from 3 experiments ± S.E.

Parameter			Control	JA-Me	NaCl	JAMe+NaCl
Dry mass	1 h	light	0.92 ± 0.02	0.91 ± 0.01	0.81 ± 0.03	0.87 ± 0.03
[g dm^{-3}]		dark	0.92 ± 0.01	0.85 ± 0.07	0.78 ± 0.02	0.86 ± 0.03
	3 h	light	0.98 ± 0.01	0.96 ± 0.03	0.67 ± 0.03	0.92 ± 0.04
		dark	0.95 ± 0.04	0.88 ± 0.04	0.61 ± 0.03	0.78 ± 0.05
	5 h	light	1.27 ± 0.09	1.01 ± 0.01	0.76 ± 0.03	0.99 ± 0.02
		dark	1.01 ± 0.02	0.86 ± 0.05	0.62 ± 0.04	0.80 ± 0.06
$^{14}CO_2$ fixation	1 h	light	37.5 ± 2.5	34.4 ± 1.8	30.2 ± 2.1	32.7 ± 2.1
[mmol($^{14}CO_2$) dm^{-3} s^{-1}]		dark	30.1 ± 3.2	28.3 ± 2.4	26.9 ± 2.2	28.1 ± 2.9
	3 h	light	29.1 ± 1.2	25.5 ± 2.1	20.1 ± 1.6	24.1 ± 1.9
		dark	27.5 ± 2.4	24.4 ± 2.6	20.5 ± 1.5	22.5 ± 1.7
	5 h	light	38.8 ± 2.2	33.1 ± 2.5	18.8 ± 1.6	24.7 ± 2.1
		dark	25.5 ± 2.2	24.7 ± 1.4	11.9 ± 1.8	18.8 ± 1.7
Proline [μg dm^{-3}]	1 h	light	79 ± 7.9	229 ± 3.6	276 ± 20.9	449 ± 23.5
		dark	108 ± 11.2	139 ± 10.8	176 ± 15.2	286 ± 13.7
	3 h	light	93 ± 9.8	259 ± 19.6	372 ± 22.7	649 ± 31.9
		dark	103 ± 9.3	153 ± 13.2	286 ± 19.4	359 ± 20.4
	5 h	light	83 ± 6.9	636 ± 47.2	972 ± 51.7	1055 ± 63
		dark	93 ± 7.3	276 ± 20.2	702 ± 49.7	825 ± 47.6
MDA [μmol g^{-1}(d.m.)]	1 h	dark	0.72 ± 0.03	0.74 ± 0.03	0.80 ± 0.05	0.76 ± 0.04
	3 h	dark	0.96 ± 0.04	1.04 ± 0.04	1.25 ± 0.06	1.09 ± 0.04
	5 h	dark	1.20 ± 0.05	1.33 ± 0.04	1.68 ± 0.08	1.42 ± 0.06
Chelators	2 min	dark	117 ± 4.6	76 ± 4.6	59 ± 2.4	64.4 ± 2.1
[μmol(HA stable Fe) g^{-1}(d.m.)]	1 h	dark	0	0	0	0
	3 h	dark	0	0	0	0
	5 h	dark	0	0	0	0

Proline content increased during the salt treatment (Table 1): the increase was nearly 10-fold after 5 h incubation in the light. The light also stimulated JA-Me-induced proline accumulation. We found synergistic effect when NaCl and JA-Me were supplied simultaneously. It has been reported that proline accumulation is a symptom of stress injury rather than an indicator of resistance (Perez-Alfocea *et al.* 1994). We suggest that treatment with 175 mM NaCl both in the light and in dark induced similar stress injury in algae, but the proline level was more than 2-fold higher in the light. The different level of endogenous proline under the same NaCl concentration raise the question of the role of proline to osmotic adjustment.

Concentrations of Na^+ and Cl^- of salt treated cells increased and JA-Me treatment did not essentially

influence its accumulation [NaCl - 1.11 g(Na^+) kg^{-1}(d.m.) and 0.99 g(Cl^-) kg^{-1}(d.m.) and NaCl + JA-Me - 0.95 g(Na^+) kg^{-1}(d.m.) and 0.89 g(Cl^-) kg^{-1}(d.m.)].

The green alga *Scenedesmus incrassatulus* Bohl. R-83 in the presence of Fe^{3+} released chelators which enhanced both solubility and availability of Fe (Benderliev and Ivanova 1996). No newly-released chelators were registered 1, 3 and 5 h after inoculation. NaCl-treated cells accumulated more MDA and released less chelators than the control (Table 1). JA-Me stimulated MDA accumulation and inhibited the Fe-induced release of chelators. JA-Me alleviated the effect of NaCl on the accumulation of MDA and on the release of chelators. The present results support a suggestion that the Fe-induced release of chelators was not caused by a leakage of organics from holes in biomembranes, because NaCl inhibited the chelator release and at the same time stimulated the accumulation of MDA. The enhanced concentration of endogenous chelators under stress conditions might be interpreted as a protective strategy of cells against iron-catalyzed generation of active oxygen. In the presence of JA-Me there is a lower need for intracellular chelators to counteract the salt stress and it might be suggested that JA-Me trigger some other protective mechanisms.

JA-Me may act as stress modulator by changing the stress responses of plants in a complex manner. With initiation of the stress the increase in JA-Me (Moons *et al.* 1997) helped the plants to face the change in the environment through stomatal closure (Satler and Thimann 1981), decreased ion accumulations (Fedina and Tsonev 1997), osmotic adjustment or synthesis of stress proteins (Ananieva and Ananiev 1999, Ali *et al.* 1999). JA-Me also affected the induction of enzymes of the lipoxygenate pathway (Blee 1998), thus accelerating the removal of oxidized lipids. In our case, JA-Me increased the content of endogenous proline but did not influence Na^+ and Cl^- accumulation in the cells. JA-Me alleviated the inhibitory effect of NaCl on the $^{14}CO_2$ fixation. The data in this study support the suggestion that JA-Me might control the lipid peroxidation affecting the intracellular pool of organic Fe chelators. It is possible that JA-Me has some other functions which enable it to be involved in the mechanisms of tolerance or suscebility of plants to salinity.

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