EFFECT OF ADDEN N-SOURCES ON SALINIZED *SCENEDESMUS OBLIQUUS*

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SUMMARY: The physiological responses of Scenedesmus to short-term NaCI salinization or to treatments with combinations of NaCI and $NaNO_3$ or NH_4CI were investigated. With the rise of NaCI salinization level in the culture medium, the photosynthetic O_2-evolution was markedly reduced whereas respiration was generally promoted. The contents of soluble carbohydrates, soluble proteins, free amino acids (in particular proline) were raised with the rise of NaCI level. The addition of $NaNO_3$ especially in small amounts resulted in a promotion of photosynthetic O_2-evolution and O_2-uptake when compared to those of the reference culture containing only NaCI as a salinizing agent. The contents of soluble proteins, amino acids, including proline also increased when compared to those of control culture, but when compared to those of reference cultures, these contents decreased with the rise of combination level. Addition of NH_4CI resulted in a suppression of photosynthetic O_2-evolution and respiration and in an elevation of the contents of soluble carbohydrates and soluble proteins and free amino acids.

Key Words: Scenedesmus obliquus, salinization.

INTRODUCTION

The surrounding environmental conditions alter the intensity and rate of plant growth. In this respect, while the photosynthetic O_2-evolution by some organisms was gradually inhibited as NaCI concentration in the medium was raised, some other organisms were found to be sodium dependent (2,9,19,20,37). However, respiration was recorded to be mostly stimulated by increasing salt concentrations (1,6,7,31,41). According to Lambers (29) this stimulation of respiration may be due to the increased availability of soluble respirable substrates, such as sugars and organic acids.

As a result of these disturbances in photosynthesis and respiration which were accompanied by alterations in dry matter production, the soluble and insoluble organic constituents were variably consumed or accumulated (11-5,18,21,25,34,36,46,48,50).

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The most abvious changes in metabolism under these drastic conditions are mainly relevant to nitrogen metabolism. That is why the question arose, whether these disturbances in nitrogen metabolism could be due to disturbances in nitrogen availability. Therefore, it was suggested that perhaps the addition of some N-sources at various concentrations may positively influence the response of plant cells to salinity.

Thus, the aim of the present investigation was to study the interactive effects of salinization and added nitrogen sources ($NaNO_3$ and NH_4Cl) on some metabolic processes of the green cells. In order to follow the alterations that might take place in plant cells early after being subjected to the various treatments, short-term experiments were conducted to clarify the direct response of the treated cells before adaptation could be established. *Scenedesmus obliquus* was used as a test organism in this study.

MATERIALS AND METHODS

In these experiments, aliquots of *Scenedesmus obliquus* cultures were subjected to two types of treatments. The first treatment was the combination of NaCl and $NaNO_3$ or NH_4Cl in the ratio of 1:1 by osmolarity and successively increasing from 00 to 200 mM. The second treatment was the use of the level 200 mM NaCl and gradually substituting NaCl by $NaNO_3$ or NH_4Cl but keeping the osmolarity constant and isoosmotic to the reference culture (containing 200 mM NaCl). The duration of the experiments was only four hours after which, the algal cultures were directly subjected to assay and analytical procedures.

Table 1: Photosynthetic O_2-evolution, respiration (O_2-uptake) and photosynthetic/respiration ratio of *Scenedesmus obliquus* cultures after being subjected for four hours (short-term experiments) to:

a) various levels of NaCl (reference cultures)

Salinization levels (mM NaCl)	O_2-evolution$^+$ % control	O_2-uptake$^+$ % control	Photosynthesis (PS)/ respiration (RES) ratio (Net PS+RES / RES)
00	100	100	5.67
50	111.45	110	5.69
100	84.00	133.5	3.92
150	73.70	146.8	3.41
200	63.15	164.2	2.77

b) various combinations of NaCl and $NaNO_3$ or NH_4Cl in the ratio of 1:1 by osmolarity, giving osmotic levels equivalent to those of reference cultures containing NaCl only.

NaCl + $NaNO_3$ (mM)	O_2-evolution		O_2-uptake		PS / RES ratio
	% cont.	% ref.	% cont.	% ref.	
00 + 00	100	-	100	-	6.42
25 + 25	126.4	113.9	127.4	110.0	6.38
50 + 50	114.0	136.3	143.3	107.3	6.31
75 + 75	87.3	117.8	159.8	110.6	3.96
100 + 100	69.2	116.7	186.0	109.7	3.02
NaCl + NH_4Cl (mM)					
00 + 00	100	-	100	-	4.91
25 + 25	85.5	76.4	121.7	116.4	3.75
50 + 50	51.1	60.6	87.4	65.1	3.29
75 + 75	30.8	42.0	73.1	49.0	2.65
100 + 100	22.5	33.6	40.6	35.3	2.57

c) various isoosmotic combinations of NaCl and $NaNO_3$ or NH_4Cl giving osmotic levels always equivalent to 200 mM NaCl.

NaCl + $NaNO_3$ (mM)	O_2-evolution		O_2-uptake		PS/RES ratio
	% cont.	% ref.	% cont.	% ref.	
00 + 00	100	156.0	100	79.1	7.23
200 + 00	64.1	100	126.4	100	4.16
150 + 50	83.9	131.0	158.2	125.2	4.31
100 + 100	70.1	109.3	166.4	131.7	3.62
50 + 150	57.5	89.8	174.6	138.1	3.05
00 + 200	47.9	74.7	170.9	135.3	2.75
NaCl + NH_4Cl (mM)					
00 + 00	100	136.1	100	72.4	6.73
200 + 00	73.4	100	138.2	100	4.05
150 + 50	41.4	56.4	119.1	86.2	2.99
100 + 100	24.9	33.9	82.7	59.9	2.73
50 + 150	13.0	17.7	49.9	35.5	2.52
00 + 200	5.2	5.6	21.8	15.8	2.38

+ μ mole of O_2 mg^{-1} dry weight h^{-1}

Photosynthesis (O_2-evolution) and respiration (O_2-uptake) were determined using the manometric technique developed by Otto Warburg as given by Umbreit *et al.* (49) and recommended Halliwell (23). Photosynthesis/respiration ratios were also calculated.

For the determination of water soluble carbohydrates the anthrone sulphuric acid method was used (17,38). Water soluble proteins were determined according to the method adapted by Lowry *et al.* (33). Proline was colorimetrically determined according to the method adopted by Bates *et al.* (59). Free amino acids were extracted from algal suspension and colorimetrically determined using the method of Moore and Stein (35).

RESULTS AND DISCUSSION

The photosynthetic oxygen evolution except at the level of 50 mM NaCl, decreased markedly with the elevation of NaCl salinization (Table 1a). The addition of $NaNO_3$ in successively increasing values (1:1 by osmolarity) resulted in an increase in photosynthetic O_2-evolution up to the level of 50 mM NaCl plus 50 mM $NaNO_3$. There-

Table 2: Soluble carbohydrate and soluble protein contents of *Scenedesmus obliquus* cultures after being subjected for four hours (short-term experiments) to:

a) various levels of NaCl (reference cultures)

Salinization levels (mM NaCl	Soluble carbohydrates$^+$ % control	Soluble protein$^+$ % control
00	100	100
50	135.5	113.0
100	152.5	113.0
150	167.0	128.5
200	205.0	136.0

b) various combinations of NaCl and $NaNO_3$ or NH_4Cl in the ratio of 1:1 by osmolarity, giving osmotic levels equivalent to those of reference cultures containing NaCl only.

NaCl + $NaNO_3$ (mM)	Soluble carbohydrates		Soluble proteins	
	% cont.	% ref.	% cont.	% ref.
00 + 00	100	-	100	-
25 + 25	137	91	104	90
50 + 50	117	88	112	89
75 + 75	170	94	118	90
100 + 100	190	84	129	91
NaCl + NH_4Cl (mM)				
00 + 00	100	-	100	-
25 + 25	146	121	135	115
50 + 50	158	115	142	113
75 + 75	171	111	146	120
100 + 100	196	107	156	121

c) various isoosmotic combinations of NaCl and $NaNO_3$ or NH_4Cl giving osmotic levels always equivalent to 200 mM NaCl.

NaCl + $NaNO_3$ (mM)	Soluble carbohydrates		Soluble proteins	
	% cont.	% ref.	% cont.	% ref.
00 + 00	100	65	100	82
200 + 00	156	100	121	100
150 + 50	141	91	114	94
100 + 100	161	103	104	85
50 + 150	170	110	89	74
00 + 200	189	122	113	93
NaCl + NH_4Cl (mM)				
00 + 00	100	53	100	87
200 + 00	189	100	115	100
150 + 50	200	106	122	105
100 + 100	211	112	129	112
50 + 150	233	124	141	122
00 + 200	222	117	168	146

+ μ mole of O_2 mg^{-1} dry weight h^{-1}

Table 3: Fre proline and other free amino acid contents of *Scenedesmus obliquus* cultures after being subjected for four hours (short-term experiments) to:

a) various levels of NaCl (reference cultures)

Salinization levels (mM NaCl	Free proline % control	Other free amino acids % control
00	100	100
50	111.5	99.5
100	223.5	102.5
150	135.5	102.5
200	153.5	92.0

b) various combinations of NaCl and $NaNO_3$ or NH_4Cl in the ratio of 1:1 by osmolarity, giving osmotic levels equivalent to those of reference cultures containing NaCl only.

NaCl + $NaNO_3$ (mM)	Free proline		Other free amino acids	
	% cont.	% ref.	% cont.	% ref.
00 + 00	100	-	100	-
25 + 25	111	97	77	82
50 + 50	118	92	71	78
75 + 75	125	90	66	77
100 + 100	143	91	60	76
NaCl + NH_4Cl (mM)				
00 + 00	100	-	100	-
25 + 25	126	103	120	107
50 + 50	136	115	127	104
75 + 75	146	110	137	106
100 + 100	164	109	151	132

c) various isoosmotic combinations of NaCl and $NaNO_3$ or NH_4Cl giving osmotic levels always equivalent to 200 mM NaCl.

NaCl + $NaNO_3$ (mM)	Free proline		Other free amino acids	
	% cont.	% ref.	% cont.	% ref.
00 + 00	100	55	100	121
200 + 00	183	100	83	100
150 + 50	179	104	73	88
100 + 100	191	111	62	74
50 + 150	205	120	85	102
00 + 200	176	103	102	123
NaCl + NH_4Cl (mM)				
00 + 00	100	62	100	113
200 + 00	161	100	89	100
150 + 50	174	108	104	117
100 + 100	189	117	132	149
50 + 150	190	118	145	163
00 + 200	178	111	154	173

+ μ g mg^{-1} dry weight.

above, the photosynthetic activity decreased again but remained higher than those of the corresponding reference cultures containing NaCl only (Table 1b). When NaCl was gradually substituted by $NaNO_3$, keeping the osmolarity of all combinations constant and equivalent to 200 mM NaCl, photosynthesis when compared to that of control culture decreased with the rise of combination level. However, these values remained higher than those of the reference culture up to the level of 100 mM NaCl and $NaNO_3$, above which it decreased again (Table 1c). In accordance with this, Grant and Canvin (22) stated that the addition of $NaNO_3$ in low concentrations to intact chloroplasts isolated from spinach resulted in transient increase in both O_2-evolution and CO_2 fixation. This

increase is apparently combined with the increase in activity of the key photosynthetic enzymes; phosphoenol pyruvate carboxylase and ribulose biphosphate carboxylase (44).

The addition of NH_4Cl obviously suppressed the photosynthetic O_2-evolution with the rise of added ammonium dose, whatever the combination (successively increasing or isoosmotic) used (Tables 1b and c). Moreover, these values were comparatively lower than those of control or reference cultures.

Respiration of *Scenedesmus obliquus* cultures salinized for four hours with NaCl, increased rapidly with the rise of salinization level. Such stimulation in respiration of NaCl salinized algal cultures is referred to as salt or maintenance respiration (6, 29, 40, 41). The productivity of green cells, could considered as the net result of photosynthesis and respiration. Therefore, the values of photosynthesis/respiration ratio are considered as a criterion for final productivity of plants. The values of this ration in the case of *Scenedesmus obliquus* decreased considerably with the rise of salinization level. This means that although productivity was negatively affected, yet the algae remained viable to overcome the drastic effect through maintenance respiration.

The addition of $NaNO_3$ (Table 1b) in a successive increase (1:1 by osmolarity) resulted also in an increase in respiration with the rise of combination level. When NaCl was gradually substituted by $NaNO_3$, but keeping the osmolarity of all combinations constant and equivalent to 200 mM NaCl (Table 1c), respiration was gradually raised with the rise of $NaNO_3$ levels when calculated on the basis of control culture. However, when calculated on the basis of reference cultures, these values remained more or less unchanged. The values of photosynthesis/respiration ratio were also lowered with the rise of combination level.

The addition of NH_4Cl (Tables 1b and c), markedly lowered the O_2-uptake when compared with those of isoosmotic corresponding reference cultures, whatever the combination (successively increasing or isoosmatic) and the calculation method (% cont. or % ret. culture) used. Also, the photosynthesis/respiration ratio exhibited lowered values, when compared with those containing only NaCl. Thus, it can be concluded that both photosynthesis and respiration were negatively affected by the addition of NH_4Cl. This was also the case whatever the combination used. In accordance with this, some authors reported that the addition of relatively mild or higher concentrations of NH_4Cl often resulted in toxic effects on respiration (28,30,32).

The contents of soluble carbohydrates as well as of soluble proteins of *Scenedesmus obliquus* cells were found to be considerably elevated with the rise of NaCl level (Table 2a). This accumulation of soluble components was repeatedly recorded under salinity conditions in various algae (16,24,43,51) and higher plants (8,10,27).

The addition of various amounts of $NaNO_3$ to the salinization medium lowered the contents of soluble carbohydrates and proteins but only when compared to the corresponding reference cultures. This means that $NaNO_3$ could increase the tolerance of algae to salinization treatments and consequently the soluble carbonhydrates and proteins (compatible solutes) were utilized for cellular demands other than stress neutralization (47). This was the case whatever the combination treatment used (Tables 2b and c). On the other hand, the addition of NH_4Cl resulted in an increase in soluble carbohydrate and soluble protein contents. This was also the case, whatever the combination treatment used. This means that the effect of added NH_4Cl was additive to the inhibitory effect of NaCl salinizing agent. These adverse alterations could be ascribed to ammonium toxicity (3).

Concerning the free amino acids, it can be seen that when *Scenedesmus obliquus* was subjected for four hours to various NaCl salinization levels the contents of free amino acids remained more or less unchanged (Table 3a). This in accordance with the results obtained by some other authors (4, 10). When *S. obliquus* cultures were subjected to various combinations of NaCl and $NaNO_3$ (Tables 3b and 3c), the contents of free amino acids were in most cases lowered when compared with the control or the corresponding reference cultures. Taking into consideration that the increase in free amino acids is an indication of stress, the addition of $NaNO_3$ could to some extent counteract the adverse effect of NaCl. However, when these cultures were subjected to various combinations of NaCl and NH_4Cl, the contents of amino acids were considerably higher than those of the control or reference cultures (Tables 3b, c). This means that NH_4Cl could be additionally toxic to some enzymes responsible for further nitrogen assimilation.

Proline, when calculated on the basis of control cultures, was fund to be markedly accumulated with the rise

of salinization level, whatever the salinization or $NaCl/NaNO_3$ or NaCl / NH_4Cl combinations used. However, when calculated on the basis of the reference cultures, proline contents were not vigorously changed. It should be pointed out that the addition of $NaNO_3$ reduced proline accumulation than the addition of NH_4Cl did. The accumulation of proline represents a temporary deviation from the normal metabolic pathways of glutamic acid to proline pathway. These results are in coincidence with those obtained by many other outhors (39, 42, 43, 45). According to Imamul-Hug and Larher (26), proline accumulation may be considered as an indication of a salt induced water deficit. Moreover, Fukutoku and Yamada (18) reported that, there is a close connection between lowered protein metabolism and proline accumulation.

Finally, it can be said that in short-term experiments and as far as the metabolic processes studied, the addition of $NaNO_3$ exerted a positive effect in counteraction of the adverse effects of salinity especially under moderate combinations, while the addition of NH_4Cl exerted mostly a negative effect, especially at relatively higher concentrations.

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