

# Extracellular Production of Absciscic Acid by Soil Algae under Salt, Acid or Drought Stress

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The common soil green algae *Chlorella vulgaris* and *Stichococcus bacillaris* increase extracellular absciscic acid (ABA) production under salt, acid or drought stress 5–10 times. Production of ABA also increases from young to senescent cultures 2–3 times.

The relationship between age of cultures and reaction to stress factors was found. Extracellular ABA levels are 1–2 times higher than cellular ones.

Possible influence of ABA to soil microbial associations, growth of plants and soil fauna is discussed from an ecological point of view.

## Introduction

The plant hormone absciscic acid (ABA) is well documented in the case of higher plants [1], it has also been described as fungal metabolite from phytopathogenic fungi [2], saprophytic fungi [3] or mycorrhizal genus *Bouletoua* [4].

ABA in mosses was found too [5]. It means it is widespread metabolite through evolution.

The statement that ABA is entirely absent in algae [5] has been challenged recently with the development of more sophisticated methods, because ABA was found in green algae [6–8] as well as in cyanobacteria [7, 9, 10].

The influence of ABA on the metabolism of lower plants is ambiguous, but there is already some knowledge about conditions and factors which can stimulate ABA production in algae and cyanobacteria [7, 9, 10].

In higher plants, the changes in ABA levels play an important role in stress physiology [1], whereas almost nothing is known about the ABA production and ABA involvement in the stress metabolism at the cellular level of organization in proto-phytes.

The aim of this study was to quantify the amount of ABA released from algal cells into the culture medium under salt, acid or drought stress.

The influence of the age of cultures on extracellular production of ABA was also investigated.

## Materials and Methods

### Plant material

Soil green algae *Stichococcus bacillaris* NAGELI strain A 408 and *Chlorella vulgaris* BEIJER strain Schwartz 75 obtained from the collection of autotrophic organisms in the Institute of Botany, Třeboň were used as experimental organisms.

The algae were cultivated in Bold's Basal Medium (BBM) [11] in suspense cultures (or in Petri dishes on agar plates in the case of drought stress) on a 12 h photoperiod ( $127 \mu\text{E m}^{-2} \cdot \text{s}^{-1}$ ) at 21 °C. The suspense cultures (600 ml in 1000 ml flask) were aerated with sterile air, the cultures in Petri dishes were grown in sterile cultivator. The fresh weight of algal biomass was established after centrifugation of the algal suspension, or in the case of cultures growing on agar the algal cells were washed by bidistilled water from agar and centrifugated. Sediment in centrifugation tubes was diluted by 5 ml of distilled water into the small test tube and fresh weight was determined by weighing. Every two days, 4 ml of sterile BBM (without

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NaCl in control or with one in the case of experimental variants) was added into the every flask by sterile syringe, because a evaporation. For each determination 1 g of biomass (fresh weight) was used.

The alga *Stichococcus bacillaris* was taken to harvesting in the end of period of intensive growth (19 days old cultures).

In the case of *Chlorella vulgaris*, we used every time both – young cultures in intensive phase of growth, 7 days old, and cultures after the end of exponential phase of growth, 28 days old, called senescent.

#### Purification procedure and ABA determination

The protocol of purification procedure involved centrifugation ( $2260 \times g$ , 20 min) of algal culture, adding to one half of the supernatant a known amount of ABA standard to determine the ABA recovery (both halves were processed in the same way), freezing of supernatant, slow thawing, further centrifugation ( $2260 \times g$ , 15 min). After adjusting pH to 2.5 by acetic acid the supernatant was passed through a preconditioned (with methanol, water and 0.2 M acetic acid) Sep-Pak C18 cartridge (Millipore Waters, U.S.A.).

The cartridge was washed with 0.2 M acetic acid and 10% MeOH; ABA was eluted with 5 ml of MeOH–0.2 M acetic acid (3:2, v/v). The eluate was evaporated at 35 °C with a stream of N<sub>2</sub>, the residue was dissolved in 500 µl MeOH and used for ABA quantification by high performance thin layer chromatography (HPTLC) and scanning densitometry using TLC Scanner II by Camag (Switzerland).

The recovery of the procedure was  $94 \pm 3\%$ . More detailed description of this method was published in our previous paper [9].

#### Design of experiments

To establish the influence of acid stress on the production of extracellular ABA, pH was adjusted 4 days before harvesting to 4.5 by addition of 0.1 N H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> (2:1). To examine the influence of drought stress on the production of extracellular ABA, algae growing on BBM was cultivated in 100 Petri dishes. 50 of them were opened for 4 days in a sterile cultivator to drought. After that agar plates were extracted with bidistilled water.

The influence of salt stress on production of extracellular ABA was evaluated by addition of sodium chloride 4 days before harvesting to reach a concentration in the culture medium of 0.05 or 0.1 M NaCl.

## Results and Discussion

The amounts of extracellular ABA produced by algae under different stress conditions are summarized in Tables I and II. As it is evident from tables, both green algae can produce abscisic acid under different environmental stress conditions, as well, it is perspicuous that all kinds of stress (drought, salt and acid) increased the level of ABA in medium 5–10 times. In addition, we found the

Table I. Extracellular ABA production [ng·g<sup>-1</sup>] under drought, acid and salt (NaCl) stress. Values are means of four replications ± S.E. Significant differences ( $P < 0.01$  ANOVA, SNK-test) between control and variants are indicates by asterisks.

Treatments	<i>Stichococcus</i>	<i>Chlorella</i>	
		7 days old	28 days old
Control	25 ± 0.04	10 ± 0.03	40 ± 0.07
Drought	190 ± 0.10**	186 ± 0.10**	212 ± 0.21**
Control	26 ± 0.02	11 ± 0.03	42 ± 0.04
pH 4.5	139 ± 0.03**	151 ± 0.08**	174 ± 0.09**
Control	24 ± 0.03	12 ± 0.04	41 ± 0.05
0.05 M	89 ± 0.04**	132 ± 0.06**	156 ± 0.09**
0.1 M	140 ± 0.14**	152 ± 0.12**	181 ± 0.15**

Table II. Extracellular ABA production [ng·g<sup>-1</sup>] and cellular content of ABA [ng·g<sup>-1</sup>] 4 days after application of 0.1 M NaCl. Values are means of four replications ± S.E.

		<i>Chlorella</i>	<i>Stichococcus</i>
7 days old cultures			
Control	cells	6 ± 0.03	8 ± 0.03
	medium	11 ± 0.05	12 ± 0.02
0.1 M NaCl	cells	61 ± 0.04	52 ± 0.07
	medium	110 ± 0.12	105 ± 0.09
28 days old cultures			
Control	cells	29 ± 0.10	27 ± 0.12
	medium	45 ± 0.08	40 ± 0.08
0.1 M NaCl	cells	75 ± 0.12	86 ± 0.11
	medium	168 ± 0.19	146 ± 0.14

Table III. Growth [mg fresh weight per ml] and extracellular ABA production [ $\text{ng} \cdot \text{g}^{-1}$ ] of cultures *Chlorella vulgaris* and *Stichococcus bacillaris* during 28 days of cultivation. Values are means of four replicates  $\pm$  S.E.

Days of cultivation	<i>Chlorella</i>		<i>Stichococcus</i>	
	Growth	ABA	Growth	ABA
7	$0.75 \pm 0.04$	$8 \pm 0.05$	$0.96 \pm 0.08$	$13 \pm 0.05$
14	$5.50 \pm 0.12$	$20 \pm 0.11$	$4.20 \pm 0.13$	$21 \pm 0.11$
21	$5.00 \pm 0.10$	$31 \pm 0.12$	$3.70 \pm 0.11$	$26 \pm 0.15$
28	$4.49 \pm 0.23$	$33 \pm 0.16$	$3.50 \pm 0.27$	$39 \pm 0.19$

enhancement of ABA level during the growth from young culture to the senescent one (Table III).

From our experiments, however, it is perceptible that extracellular ABA production is 1–2 times higher than the cytoplasmatic one (Table II). A similar trend was found in the case of cyanobacteria [10]. In the work of Hirsch *et al.* [8], the cytoplasmatic ABA content is mostly determined, but a high extracellular ABA production is also mentioned.

Of course, at present this phenomenon is questionable, but preliminary results [9, 10] and our presented findings of high extracellular ABA production show, that the releasing of ABA seems to be not only the diffusion from cells into the medium, but an active transport across membranes (because there are in the cells lower ABA levels than in the medium). This extracellular production should have an ecological significance.

The extracellular ABA produced by algae under all kinds of stress can already be found 4 h after stress application, as well as in the case of cyanobacteria [10]. However, the quantity of ABA released from green algae was higher than from cyanobacteria.

Among factors, which can influence the response of stressed organisms, as duration of stress period, cultivation conditions or intensity of stress factors [10], the age of cultures is also significant (see Tables I–III). It may play a specific role in the case of interactions between environmental stress factors (drought-heat; cold-light deprivation; salinity-dessication *etc.*) [12], which are common in nature. Algae seem to be very suitable experimental organisms for these experiments.

The stimulation of extracellular production of ABA from senescent and at the same time stressed cultures in the case of *Chlorella vulgaris* (Table I) shows, that older cultures are able to produce higher amount of ABA. This is very important from the ecological point of view, because young, senescent and stressed algal communities commonly live together. ABA is produced in plants as a defensive antistressing metabolite and also induces the synthesis of novel specific proteins which might play a special role in an acquired stress tolerance [13]. Moreover, in the soil conditions besides algae and cyanobacteria, fungi living in the soil can produce a large amount of ABA [2–4]. Of course, extracellular ABA produced by soil algae, cyanobacteria or fungi can influence not only microbial associations, physiological activity of roots, growth of higher plants, but can also influence life cycles of some invertebrates. ABA can affect the metabolic activity of soil insecta and fecundity and egg viability of aphides [14].

From soil microbial processes, for example, the nitrogenase activity is influenced by ABA. ABA can stimulate nitrogenase activity of bacteria and cyanobacteria [16, 17]. These findings extend our knowledge about possible influence of soil autotrophic organisms on some microbiological, biochemical and physical soil properties.

Absciscic acid in soil conditions can act as a natural herbicide [15]. Due to ABA, its satellite metabolites and synthetic analogues having herbicidal activity and the influence on insects they could be used in the future as metabolizable pesticides.

The ability of ABA production by other algae and possibility of affection of other soil organisms by ABA at present being investigated.

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