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BRIEF COMMUNICATION

EXPERIMENTAL DATA FROM THREE NATIVE REPRESENTATIVES OF NATURAL COMMUNITIES IN NORTH-EAST RUSSIA: DOES THE ACTIVITY OF THE ALTERNATIVE RESPIRATORY PATHWAY IN LEAVES DEPEND ON THE CARBOHYDRATE STATUS?

Natalia V. Pystina, Roman A. Danilov*

Department of Natural & Environmental Sciences, Mid Sweden University, S-87188 Härnösand, Sweden

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Summary. The relationship between respiratory alternative pathway and carbohydrate status was studied in mature leaves of three native representatives of forest and meadow communities of north-east Russia – *Ajuga reptans* L., *Achillea millefolium* L. and *Calla palustris* L. All experiments were carried out on plants growing in natural habitats. The activity of the alternative respiratory pathway was studied using method of specific inhibitors. 25.0 mmol benzhydroxamic acid (BHAM) was used as inhibitor of alternative oxidase (AOX). Our results showed a clear positive relationship between the activity of the alternative respiratory pathway and carbohydrate content in the leaves of the plants studied. The possibile regulation of the activity of alternative pathway by carbohydrate concentrations in mature leaves is discussed.

Key words: alternative oxidase, alternative respiratory pathway, carbohydrates, leaves, respiration

Abbreviations: AOX – alternative oxidase, BHAM – benzhydroxamic acid, COX – cytochrome oxidase

Introduction

Respiration is a key process providing plants with energy and metabolites. Respiratory activity in plants has been shown to be sensitive to environmental factors. One of the

* Corresponding author, Tel: + 46 611-86292, Fax: + 46 611-86010, e-mail: Roman.Danilov@tnv.mh.se

important functions of respiration is its protecting role against stress (Larcher, 1995). The respiratory electron transport consists of cytochrome (cyanide-sensitive) and alternative (cyanide-resistant) pathways. Cytochrome oxidase (COX) acts as the key enzyme of the electron transport chain. Thereby, the cytochrome pathway is the main source of ATP in plants. On the other hand, the alternative pathway is generally believed not to produce energy in the form of ATP. Although the alternative pathway can considerably contribute to plants energy balance, its physiological role still remains unclear (Vanlerberghe and McIntosh, 1997).

There are some evidence showing that the alternative pathway can play some regulatory role in carbohydrates balance (Azcon-Bieto et al., 1983; Lambers et al., 1996). However, the data are scarce and do not allow any clear conclusions. All published results have been obtained from plants grown under laboratory conditions. No data from plants studied in their natural habitats have been reported.

In the present study, we investigated relationship between the alternative pathway and carbohydrate status in leaves of three native representatives of forest and meadow communities of north-east Russia – *Ajuga reptans* L., *Achillea millefolium* L. and *Calla palustris* L. All experiments were carried out on plants growing in natural habitats.

Materials and Methods

A. reptans (Lamiaceae), *A. millefolium* (Asteraceae) and *C. palustris* (Araceae) are native representatives of natural habitats in north-east Russia (62° 52′ N near Syktyvkar). All plants were studied in July under natural conditions: *A. reptans* ("shade plants") and *C. palustris* growing under forest canopy, and *A. millefolium* growing in meadow communities. Only mature leaves were taken into consideration. The leaves were continuously sampled from 15 plants of each species at 9.00 a.m. in order to avoid any possible artefacts caused by photosynthesis metabolites. Additionally, plants of *A. reptans* were grown in open locations ("sun plants", 100% of the daylight) and studied as described above.

Small peaces (0.0028 dm^2) were cut off from the leaves sampled (three peaces per leave). The peaces were placed in a cuvette and the respiration was estimated by oxygen consumption (μmol $O_2.g^{-1}FW.h^{-1}$) with the aid of an electrode of Clark-type at 20 °C. Activity of the alternative respiratory pathway was studied using method of specific inhibitors (Bahr and Bonner, 1973; Theologis and Laties, 1978; Møller et al., 1988). 25.0 mmol benzhydroxamic acid (BHAM) was used as inhibitor of alternative oxidase (AOX). All experiments were carried out in triplicate. Activity of the alternative pathway (v_{alt}) was calculated as the difference between total respiration (in the absence of inhibitors) and respiration in the presence of 25.0 mmol BHAM.

Content of soluble carbohydrates was measured according to Sweely et al. (1963). The results were expressed as mg g^{-1} FW.

Statistical analyses were performed in the computer package Minitab 13.0.

Results and Discussion

Effects of AOX inhibitor (BHAM) on respiration in mature leaves of plants species tested are shown in Table 1. Sun plants of *A. reptans* as well as *C. palustris* exhibited highest sensitivity to BHAM. Treatment with the inhibitor led to a decline in respiration by 37–38 %, which corresponds to the activity of the alternative pathway. The activity of the alternative pathway was measured by 18% in the shade plants of *A. reptans* and by 21% in *A. millefolium*, respectively.

Table 1. Effects of alternative oxidase inhibitor BHAM on respiration (μmol $O_2.g^{-1}$ FW.h^{-1}) in mature leaves of plants from natural habitats.

Plants	Control	BHAM
C. palustris	12.9±0.9	8.0±0.9*
A. millefolium	12.1±0.6	9.6±0.4*
A. reptans (“sun plants”)	18.3±0.9	11.6±0.9*
A. reptans (“shade plants”)	8.7±0.5	7.1±0.4*

* The difference is statistically significant ($P \leq 0.05$) compared to the control. The values of SE are shown.

Higher contents of soluble carbohydrates were detected in the mature leaves of sun plants of *A. reptans* (Table 2). Relative high concentrations of carbohydrates were also observed in the leaves of *C. palustris*. The leaves of *A. millefolium* and shade plants of *A. reptans* were characterised by considerably lower concentrations of carbohydrates.

Our results showed clear positive relationship between the activity of the alternative respiratory pathway and carbohydrate content in the leaves of the plants studied.

Table 2. Content of soluble carbohydrates (mg g^{-1} FW) in mature leaves of plants from natural habitats. The values of SE are shown.

Plants	Carbohydrates
C. palustris	17.1±1.4
A. millefolium	5.8±0.4
A. reptans (“sun plants”)	30.1±1.7
A. reptans (“shade plants”)	7.3±0.3

According to an "overflow concept" (Lambers, 1982), the alternative pathway can regulate concentrations of sugars in cells when the cytochrome pathway becomes saturated or restricted. A positive relationship between content of carbohydrates and activity of the alternative pathway has been detected in some previous experiments (Azcon-Bieto et al., 1983; Rychter et al., 1988; Lambers et al., 1996). On the contrary, other authors have reported that no such relationship could be observed (e.g. Millenaar et al., 2000). However, only plants grown under optimal laboratory conditions were used as objects in the studies mentioned. Therefore, one can assume that relationships, which could be of importance in natural habitats, were masked by excessive amounts of carbohydrates. Our attempt was to study plants from natural habitats. The results obtained let us to suppose that activity of the alternative pathway in mature leaves can be regulated by carbohydrate concentrations. Increasing amounts of sugars lead to an increase in the activity of the alternative pathway. As a result, excessive carbohydrate concentrations can be avoided. This could be of importance to mature plant organs, which have ceased their growth and therefore do not need respiratory substrates.

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