

BOTÂNICA E FISILOGIA VEGETAL

NOTA

TEMPERATURE-DEPENDENT ALTERATIONS OF RESPIRATION IN LEAVES OF TWO SELECTED VASCULAR PLANT SPECIES: THE ROLE OF THE ALTERNATIVE OXIDASE PATHWAY⁽¹⁾

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ABSTRACT

Effects of higher temperatures on respiration and activity of alternative oxidase (AOX) were studied in mature leaves of *Ajuga reptans* L. and *Rhodiola rosea* L. Total respiration in both *A. reptans* and *R. rosea* increased exponentially with the increasing temperature of 10 °C to 35 °C. Respiration in the presence of benzhydroxamic acid (BHAM) also increased exponentially in accordance with the increasing temperature in the leaves of both *A. reptans* and *R. rosea*. Relative activity of the alternative pathway decreased significantly in the leaves of *A. reptans* with increasing temperatures. However, an increase in the relative activity of the alternative pathway was detected in the leaves of *R. rosea*. Thermoresistance of the alternative respiratory pathway was considered to be higher in *R. rosea* compared to *A. reptans*. We suppose that *A. reptans* and *R. rosea* have different mechanisms regulating partitioning of electrons to the alternative respiratory pathway.

Key words: alternative respiratory pathway, respiration, alternative oxidase, leaves, temperature.

RESUMO

ALTERAÇÕES DE RESPIRAÇÃO DEPENDENTES DE TEMPERATURA EM FOLHAS DE DUAS ESPÉCIES DE PLANTAS VASCULARES SELECIONADAS: O PAPEL DO CAMINHAMENTO ALTERNATIVO DA OXIDASE

Estudaram-se os efeitos de temperaturas mais altas na respiração e na atividade do caminhamento alternativo da oxidase (AOX) em folhas maduras de *Ajuga reptans* L. e de *Rhodiola rosea* L. Verificou-se que a respiração total, tanto em *A. reptans* como em *R. rosea*, apresentou aumento exponencial com a temperatura crescente de 10 °C para 35 °C. Observou-se, também, que na presença de ácido benzidroxâmico (BHAM) houve aumento exponencial na respiração com a variação crescente da temperatura nas folhas das duas plantas estudadas. A atividade relativa do caminhamento alternativo diminuiu significativamente nas folhas de *A. reptans* com temperaturas crescentes; verificou-se, porém, aumento nas folhas de *R. rosea*. A termorresistência do caminhamento respiratório alternativo foi considerada mais alta em *R. rosea* que em *A. reptans*. Supõe-se que ambas possuem diferentes mecanismos que regulam a partição de elétrons para o caminhamento respiratório alternativo.

Palavras-chaves: caminhamento respiratório alternativo, respiração, oxidase alternativo, folhas, temperatura.

Temperature is considered to be one of the most important environmental factors affecting all physiological processes in plants. Respiration is one of the key processes in plants providing them with energy and metabolites. General relations between temperature and respiration are well known (LARCHER, 1995). However, reports on the influence of temperature on the alternative respiratory pathway in vascular plants are really scarce (VANLERBERGHE and McINTOSH, 1997). A cytochrome pathway (cyanide-sensitive) and an alternative

pathway (cyanide-resistant) are considered to be principal parts of the respiratory chain in plant mitochondria. The alternative pathway consists of only one enzyme – alternative oxidase (AOX) and is generally considered to be nonphosphorylating (e.g. McINTOSH, 1994). There are some evidences that the alternative pathway plays a role in thermogenesis in aroids and other species (MEEUSE, 1975). However, the role of AOX in non-thermogenic tissues of vascular plants remains still unclear (WAGNER and KRAB, 1995). As stated by numerous authors,

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alternative pathway is an important feature of respiration in plants and, therefore, new evidences contributing to our knowledge of regulatory mechanisms of the alternative pathway are urgently needed (for an excellent review see VANLERBERGHE and MCINTOSH, 1997).

There are some reports in the literature dealing with influence of low temperatures on the AOX activity (VANLERBERGHE and MCINTOSH, 1997, GONZALEZ-MELER et al., 1999). On the other hand, knowledge of effects of increasing temperature on AOX activity under natural conditions is of a particular interest. We studied how activity of AOX depends on increasing temperature in mature leaves of *Ajuga reptans* L. and *Rhodiola rosea* L. – two common species in natural plant communities in Komi Republic, Russia. *A. reptans* was chosen as the study object because it is a common shade-enduring habitant in European natural forest communities. We have investigated *A. reptans* from their northern areal. In this area *A. reptans* is one of the few postglacial relicts. *R. rosea* has its importance as well-known

medical plant. The choice of *R. rosea* was justified by pure knowledge of temperature-mediated effects on this important medical plants.

Material and Methods

All experiments with both *A. reptans* and *R. rosea* were carried out under natural conditions with sufficient light supply near Syktyvkar (62° 52' N), northeast Russia. Only mature (July) leaves of *A. reptans* and *R. rosea* were investigated. The leaves were continuously sampled from 15 plants at 9.00 a.m. in order to avoid any possible artefacts caused by photosynthesis metabolites.

Small pieces (0.0028 dm²) were cut off from sampled leaves (three pieces per leaf). The pieces were placed in a cuvette and the respiration was estimated by oxygen consumption (mmol O₂ g⁻¹FW h⁻¹) with the aid of an electrode of Clark-type at following temperatures: 10°, 20°, 25° and 35° C.

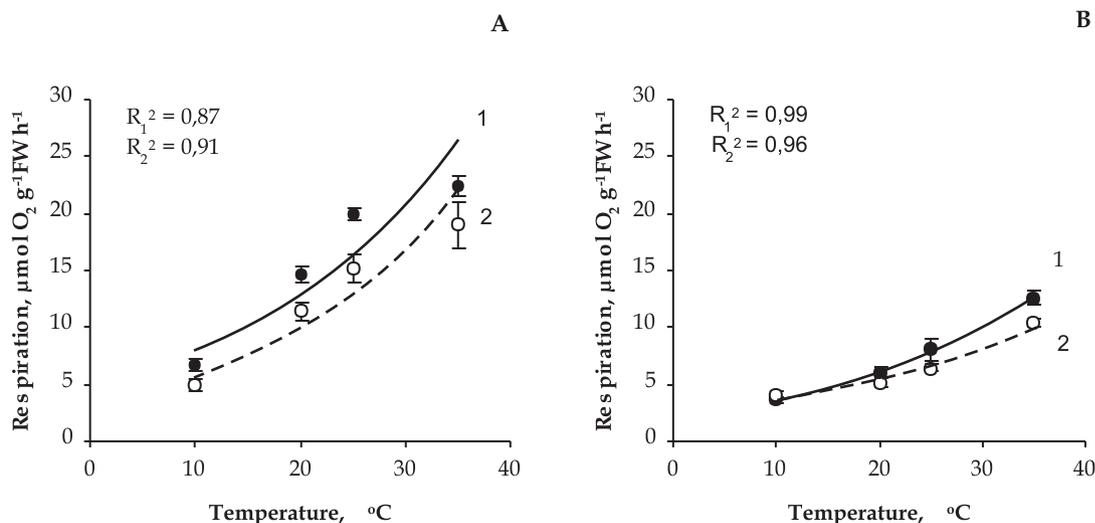


Figure 1. Effects of enhancing temperatures on respiration in mature leaves of *Ajuga reptans* (A) and *Rhodiola rosea* (B). 1: total respiration in the absence of the inhibitor of alternative oxidase. 2: respiration in the presence of the inhibitor of alternative oxidase (25.0 mmol BHAM). The values of SE are shown.

Table 1. Influence of increasing temperature on the activity of the alternative pathway (% compared to the total respiration) in mature leaves of *Ajuga reptans* and *Rhodiola rosea*

Temperature, °C	10	20	25	35
	%			
<i>A. reptans</i>	27.2 ± 1.9	23.2 ± 1.0	21.2 ± 1.2	16.9 ± 2.1
<i>R. rosea</i>	0	8.7 ± 0.5	13.8 ± 1.1	23.2 ± 1.5

Activity of the alternative respiratory pathway (v_{alt}) was studied using method of specific inhibitors (BAHR and BONNER, 1973; THEOLOGIS and LATIES, 1978) and was estimated as the difference between total respiration (without inhibitors) and respiration in the presence of benzhydroxamic acid (BHAM, 25.0 mmol). All experiments were carried out in triplicate.

Statistical analyses were performed in the computer package Minitab 13.0.

Results and Discussion

Total respiration in the mature leaves of both *A. reptans* and *R. rosea* increased exponentially with the increasing temperature of 10 °C to 35 °C (Fig. 1). By increase in temperature, temperature coefficient (Q_{10}) decreased from 2.2. to 1.5 in the leaves of *A. reptans* and from 2.2 to 1.6 in the leaves of *R. rosea*. Exponential behaviour of the respiration curves obtained can be considered as an evidence of a general absence of a profound stress in *A. reptans* and *R. rosea* in the temperature range studied (CRIDDLE et al., 1997). These facts correspond well with the actual knowledge concerning general dependence of respiration on temperature (e.g. Larcher, 1995). Respiration in the presence of BHAM also increased exponentially in accordance with the increasing temperature in the leaves of both *A. reptans* and *R. rosea*. Consequently, this phenomenon can be mainly explained by the activation of the cytochrome respiratory pathway which is resistant to BHAM.

Relative activity of the alternative pathway decreased significantly in the leaves of *A. reptans* with increasing temperatures. However, an increase in the relative activity of the alternative pathway was detected in the leaves of *R. rosea* (Tab. 1). We suppose that *A. reptans* and *R. rosea* have different mechanisms regulating partitioning of electrons to the alternative respiratory pathway. Previous studies showed a general increase in AOX protein concentration and activity of the alternative pathway in plant tissues at lower temperatures (VANLERBERGHE and MCINTOSH, 1992; ITO et al., 1997). However, data on the influence of higher temperature on the activity of AOX are more than scarce. A decline in the sensitivity of respiration to inhibitors of AOX was reported in the temperature range of 35–45 °C (Chauveau et al., 1978). GONZALEZ-MELER et al. (1999) reported an increase in the activities of both main and alternative pathway respiration at increasing temperatures up to 35 °C. Our results show that the sensitivity of respiration to the inhibitor of AOX (BHAM) increased exponentially according to the enhancing

temperatures in both *A. reptans* and *R. rosea*. This phenomenon is controversial to that reported by Chauveau et al. (1978). However, the relative partitioning of electrons to the alternative pathway decreased in the leaves of *A. reptans* and increased in the leaves of *R. rosea* when temperatures increased.

The role of AOX in vascular plants is still under debate (VANLERBERGHE and MCINTOSH, 1997). It is being discussed if the alternative respiratory pathway can act as one of the important mechanisms acting by chilling acclimatisation (e.g. MOYNIHAN et al., 1995). However, there is currently no reasonable hypothesis dealing with effects of higher temperatures on the activity of AOX. Our results let us postulate thermolability of respiration at higher temperature in two species studied. Partitioning of electrons to the alternative pathway seems to be considerably varying both in *A. reptans* and *R. rosea*. We suppose that the alternative respiratory pathway is more thermoresistant in *R. rosea* compared to *A. reptans*. However, our results do not allow any reasonable speculations concerning a possible role of the alternative respiratory pathway at higher temperatures in the species studied.

Conclusions

Total respiration in mature leaves of both *A. reptans* and *R. rosea* increased exponentially with the increasing temperature of 10 °C to 35 °C. With increasing temperatures, relative activity of the alternative pathway decreased significantly in the leaves of *A. reptans* while increased in the leaves of *R. rosea*. Thermoresistance of the alternative respiratory pathway can be considered to be higher in *R. rosea* compared to *A. reptans*. Different mechanisms regulating partitioning of electrons to the alternative respiratory pathway can be a valuable explanation to the phenomena detected.

References

- BAHR, J.T.; BONNER, W.D.JR. Cyanide-insensitive respiration. *Journal of Biology and Chemistry*, v.248, p.3441-3450, 1973.
- CHAUVEAU, M.; DIZENGREMEL, P.; LANCE, C. Thermolability of alternative electron transport pathway in higher plant mitochondria. *Physiologia Plantarum*, Copenhagen, v.42, p.214-220, 1978.
- CRIDDLE, R.S.; SMITH, B.N.; HANSEN, L.D. A respiration based description of plant growth responses to temperature. *Planta*, Heidelberg, v.201, p.441-445, 1997.

- GONZALES-MELER, M.A.; RIBAS-CARBO, M.; GILES, L.; SIEDOW, J.N. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. *Plant Physiology*, Sofia, v.120, p.765-772, 1999.
- ITO, Y.; SAISHO, D.; NAKAZONO, M.; TSUTSUMI, N.; HIRAI, A. Transcript levels of tandem-arranged alternative oxidase genes in rice are increased by lower temperature. *Gene*, Amsterdam, v.203, p.121-129, 1997.
- LARCHER, W. *Physiological plant ecology*. Berlin: Springer Verlag, 1995, 506p.
- MCINTOSH, L. Molecular biology of the alternative oxidase. *Plant Physiology*, Sofia, v.105, p.781-786, 1994.
- MEEUSE, B.J.D. Thermogenic respiration in aroids. *Annual Review of Plant Physiology and Plant Molecular Biology*, Palo Alto, v.26, p.117-126, 1975.
- MOYNIHAN, M.R.; ORDENTTLICH, A.; RASKIN, I. Chilling induced heat evolution in plants. *Plant Physiology*, Sofia, v.108, p.995-999, 1995.
- THEOLOGIS, A.; LATIES, G.G. Relative contribution of cytochrome mediated and cyanide-resistant electron transport in fresh and aged potato slices. *Plant Physiology*, Sofia, v.62, p.232-237, 1978.
- VANLERBERGHE, G.G.; MCINTOSH, L. Lower growth temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. *Plant Physiology*, Sofia, v.100, p.115-119, 1992.
- VANLERBERGHE, G.G.; MCINTOSH, L. Alternative oxidase: from gene to function. *Annual Review of Plant Physiology and Plant Molecular Biology*, v.48, p.703-734, 1997.
- WAGNER A.M.; KRAB K. The alternative respiration pathway in plants: role and regulation. *Physiologia Plantarum*, Copenhagen, v.95, p.318-325, 1995.