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.
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 inhabitant 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.

![Graph A](image1)

![Graph B](image2)

**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   |
|----------------|------|------|------|------|
|                | %    |      |      |      |
| A. reptans     | 27.2 ± 1.9 | 23.2 ± 1.0 | 21.2 ± 1.2 | 16.9 ± 2.1 |
| R. rosea       | 0    | 8.7 ± 0.5 | 13.8 ± 1.1 | 23.2 ± 1.5 |

![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.](image3)
Activity of the alternative respiratory pathway ($v_{aox}$) 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. 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, 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.