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Microcalorimetric evaluation of metabolic heat rates in coffee (*Coffea arabica* L.) roots of seedlings subjected to chilling stress

Cristina G.S. Queiroz[a,*], Marcos L. Mares-Guia[a], Antonio C. Magalhães[b]

[a]*Departamento de Botânica e Departamento de Bioquímica e Imunologia, ICB, UFMG, 31270-110 Belo Horizonte, MG, Brazil*
[b]*Departamento de Fisiologia Vegetal, IB, UNICAMP, 13083-970 Campinas, SP, Brazil*

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Abstract

In numerous tropical and sub-tropical plant species, such as coffee, exposure to low temperatures can cause extensive tissue damage, the seedlings being particularly sensitive to chilling stress. This condition usually induces changes in the metabolic rates, and there are indications that this process can be evaluated by monitoring heat evolution by microcalorimetry. We studied the responses of coffee seedlings to chilling stress by measuring root growth and metabolic heat rates in apical root segments of coffee seedlings exposed for 6 days to temperatures ranging from 5 to 25°C. The metabolic heat rates were measured in a heat conduction calorimeter. Root growth was progressively hindered as the seedlings were exposed to temperatures below 15°C; low temperature-induced growth inhibition was closely correlated with the lowering of metabolic heat rates. An Arrhenius plot of metabolic heat rate revealed a break in the line at 15°C, suggesting the occurrence of a metabolic transition at this temperature. The microcalorimetric technique provides a sensitive, non-invasive method for evaluating plant growth responses to chilling stress.

Keywords: Chilling stress; Growth; Plant calorimetry; Temperature coefficient

1. Introduction

Chilling sensitivity is an important issue in plant ecophysiological studies, particularly in tropical and subtropical species which show characteristic damage symptoms when subjected to chilling temperatures [1]. Chilling susceptibility limits seasonal growth, geographic distribution and storage conditions of many cultivated plants and their products [1]. The coffee plant (*Coffea arabica* L.) is a chilling sensitive species [2,3], originating from the highlands of southern Ethiopia, a region where average annual temperatures between 18.5 and 21.5°C normally prevail [4]. Studies on coffee plants reveal that temperatures below 16°C cause inhibition of vegetative growth and decreased net photosynthesis [5,6] in addition to poor bean maturation, drastically affecting plant productivity [7].

Measurement of metabolic heat rate by microcalorimetry is a sensitive and non-invasive method for monitoring plant responses to stresses such as salinity, aluminum toxicity, atmospheric pollution and extremes of temperature [8,9]. The present research investigates susceptibility of coffee roots to chilling temperatures by measuring alterations in metabolic heat rate.

* Corresponding author.
E-mail address: queiroz@mono.icb.ufmg.br (C.G.S. Queiroz)

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2. Experimental

2.1. Growth conditions

Coffee seeds (*C. arabica* L.) cv. Catuaí Vermelho were provided by the Plant Genetics Section, Agronomic Institute of São Paulo, Campinas, SP, Brazil.

After removing the fruit endocarp (parchment), the seeds were left to imbibe distilled water for 48 h at 25°C, and sown in 1 l volume plastic pots filled with vermiculite. The plants were raised in growth cabinets, in the dark, at 25°C. Water was regularly supplied by irrigation twice a week. The experiments consisted of the exposure of 30-day-old-seedlings (match stage) to continuous temperatures of 5, 10, 15, 20 and 25°C, for 6 days in darkness.

2.2. Growth measurements

To identify the zone of maximum elongation, the primary roots of 30-day-old seedlings were marked with indelible India ink, at 1 mm intervals starting from the tip. Measurements of the distances between markings allowed calculation of the rate of radicle expansion in the various temperature treatments. Further, different sets of plants were exposed for 6 days to continuous temperature treatments. Afterwards, the seedlings were transferred to the control temperature (25°C), and the capacity for resuming growth was measured.

2.3. Microcalorimetry

The calorimetric analyses were carried out in a heat conduction, batch-mixing calorimeter [10,11]. The calorimeter mixing vessels (built of gold) have a total volume of 5 ml. Each vessel has two linked compartments holding 1.0 and 3.0 ml, respectively, which are connected to thermopiles with conversion factors of 7 $\mu W\ \mu V^{-1}$.

All the calorimetric analyses were made at 25°C. The calorimeter was calibrated by the Tris reaction with HCl ($\Delta H^0 = -47.28$ kJ/mol H^+ neutralized, at 25°C [12]). Standard calibration plot obtained was $Y = 1.30150 + 0.99307x$ ($R^2 = 0.9957$), where Y is the integrated area under the output curve and x the amount of heat released.

To perform the calorimetric analyses on root segments, 1 ml of phosphate buffer (0.2 M, pH 7.4), was injected in each of the vessels of the calorimeter. A stable base line, whose development was followed up for 60 min, was obtained after the system had reached equilibrium. Data were registered at 1 s intervals. After this, apical 1 mm root segments were cut, immediately weighed (30 mg of fresh weight), introduced into a catheter with a small buffer volume, and injected into the sample vessel. The vessel was immediately closed and the same buffer volume added to the reference vessel. A period of ca. 15 min was necessary to reach a new thermal equilibrium, characterized by a steady-state in the production of heat by the sample cell.

Manual rotation of the block was done at 3 min intervals to keep the concentration of dissolved oxygen at constant levels. Average rate of metabolic heat was estimated based on the different regions of the thermogram characterized by a steady-state after each mixing. The data were reported as heat flow at steady-state level during 30 min and the results expressed as $\mu W\ mg^{-1}$ (FW) (Fig. 1).

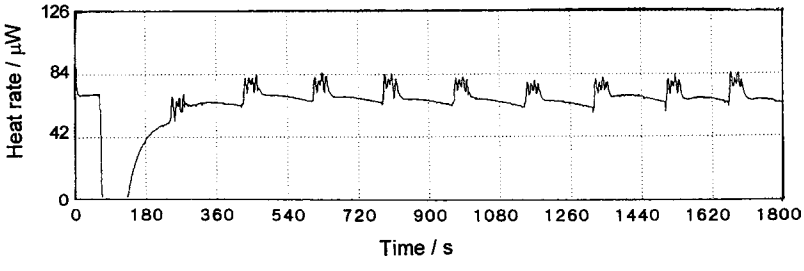

Fig. 1. Heat production by 30 mg (fresh weight, FW) of apical root segments of seedlings grown at 25°C. Thermogram showing mixing at 3 min intervals.

All the calorimetric analyses were carried out at 25°C on root segments of seedlings exposed to temperatures of 10, 12.5, 15, 20, 22.5 and 25°C for 6 consecutive days.

The value of the Arrhenius temperature coefficient (μ) [13,14] was obtained from the slope of a plot of log heat rate versus T^{-1}, where T is the absolute temperature.

3. Results

3.1. Growth measurements

The chilling treatments at 5 and 10°C imposed during six consecutive days completely inhibited root growth at 25°C, but root expansion was only partially affected by exposure to 15 and 20°C, compared to the control at 25°C (Fig. 2). As chilled plants returned to the control temperature, the 15 and 20°C exposed seedlings were capable of resuming growth, but no growth was detected in the 10°C-treated plants.

3.2. Microcalorimetry and growth

Metabolic heat rates were severely reduced in coffee seedlings exposed to temperatures below 15°C, and reached very low values in those exposed to 10°C (Fig. 3). Our data suggest a metabolic transition around 15°C, associated with chilling damage in root tissues. The Arrhenius temperature coefficient, μ obtained from the Arrhenius plot (Fig. 3) in the range of 10–15°C (μ=14,100 K) was higher (ca. 4 fold) than in the range of 15–25°C (μ=3540 K).

The curve presented in Fig. 4 shows a very close correlation between rates of heat evolution and root-tip growth over the whole range of temperatures tested. In seedlings subjected to 10°C, growth was completely inhibited, but the metabolic heat rate at 25°C was 0.66 μW mg^{-1} (FW.), i.e. 27% of the control at 25°C (2.41 μW mg^{-1} FW).

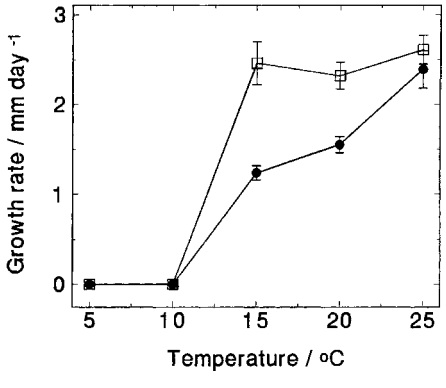

Fig. 2. Root growth of 30 day old coffee seedlings at 25°C after exposure to chilling treatments for 6 days (filled circles) and after transfer to 25°C for 6 days more (open squares). Values are means of 10 replicates. Vertical bars indicate standard deviation of the mean.

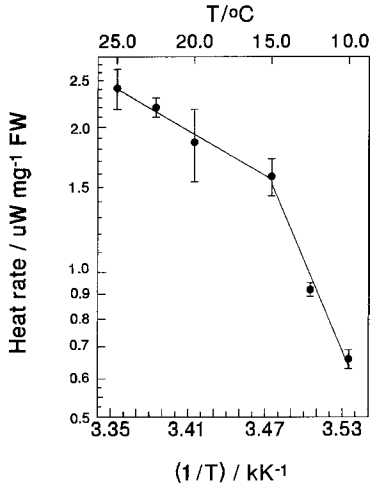

Fig. 3. Arrhenius plot of metabolic heat rates measured at 25°C of roots excised from seedlings exposed to chilling temperatures for 6 days. The values of the Arrhenius temperature coefficients are: μ=3540 K (for the range 15–25°C) and μ=14,100 (for the range 10–15°C).

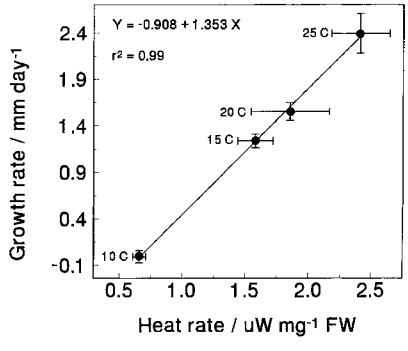

Fig. 4. Correlation between metabolic heat rate in root tissues measured at 25°C and root growth in seedlings exposed to chilling temperatures.

4. Discussion

Our data on coffee root tips show that these tissues are particularly sensitive to chilling temperatures in the range below 15°C. At 5 and 10°C root growth was completely inhibited and was accompanied by a large reduction in the metabolic heat rate. This confirms literature data on the sensitivity of coffee to low temperatures, and points out the possibility that measurement of metabolic heat rate of young seedlings might constitute an easy viability test for monitoring a variety of physiological processes associated with stress conditions.

The capability of coffee seedlings for regrowth at 25°C was highly dependent on the previous conditions to which plants were subjected. Thus, regrowth measurements indicate irreversible temperature damage at temperatures below 15°C within 6 days.

The reduction in metabolic heat rate was particularly accentuated at temperatures below 15°C, indicated in an Arrhenius plot by a temperature dependent transition between 12.5 and 15°C, with large increases in μ (Arrhenius temperature coefficient) below 15°C. A similar behavior was observed by Criddle et al. [15] in cell cultures and apical segments of tomato plants. These authors studied temperature effects on the metabolic heat rates of cultured cells and tissue segments of tomatoes and carrots. The data, plotted in Arrhenius form, showed discontinuities or abrupt changes in slope at critical temperatures. The slope change noted in the 11 to 14°C range for chilling sensitive tomato preparations was notably absent in the insensitive carrot tissue. Similarly, *Larix laricina*, a conifer that occurs in northern hemisphere, and characterized as being chilling tolerant, did not show any discontinuity in the Arrhenius curves, from 1 to 30°C, as evaluated from DSC [16].

Sudden transitions observed by microcalorimetry in chilling sensitive species might be attributed to a combination of two factors: (a) structural membrane alterations, mainly affecting the lipid fraction, and (b) increases in the activation energy of enzyme reaction, which can be associated to phase changes in the membranes [17]. Recent studies in our laboratory showed that roots of coffee seedlings subjected to chilling temperatures presented functional membrane alterations associated to lipid peroxidation, decreased membrane fluidity and, consequently, increases in electrolyte leakage [3]. These changes, detected by chemical analysis and EPR spectroscopy, occurred at the same range of temperature, between 10 and 15°C as detected by microcalorimetry. These results may indicate that the sudden fall of metabolic heat rate, after exposure of the seedlings to low temperature, is indicative of functional, altered membranes, possibly associated to the respiration process. Several researchers working with chilling susceptible species, also identified discontinuities in Arrhenius plots of cell respiration [18], such as succinate oxidase, in *Vigna radiata* [19] and various dehydrogenases, in soybean [20].

The rates of heat production in coffee roots exposed to different temperatures showed a straight correlation with the growth rates of plants under the same conditions (Fig. 4). Hansen et al. [21] measured the heat output in apical meristems of various clones of *Sequoia sempervirens*, and observed a linear correlation between the growth rate of 60-day-old unrooted clones and 25-year-old trees.

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