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BRIEF COMMUNICATION

Respiration rate and chemical composition of *Karwinskia* roots as affected by temperature

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Abstract

Two-year-old plants of *Karwinskia humboldtiana* Zucc. and *Karwinskia parvifolia* Rose grown from the seeds in greenhouse were transferred to the growth cabinet and cultivated for two months under different temperature regimes (35/20 °C - the summer temperature regime, SR, and 20/5 °C - the winter temperature regime, WR). These temperatures were similar to the temperature conditions in the natural areas of the species studied (Nuevo León, Mexico). The root respiration rate was higher in the plants cultivated under SR than in those under WR. Roots of *K. parvifolia* respired faster in both temperature regimes than roots of *K. humboldtiana.* Starch content in roots was higher in the plants cultivated under SR, however, concentrations of the other investigated organic and inorganic compounds were higher in the plants cultivated under WR. In *K. humboldtiana* roots, higher concentration of reducing sugars, carbon and hydrogen were found than in *K. parvifolia.*

Additional key words: carbon, hydrogen, nitrogen, reducing sugars, starch, summer and winter temperature regime.

Species of the genus *Karwinskia* are medicinal woody plants producing secondary metabolite peroxisomicine A_1 (anthracenone) with antineoplastic effect on the mammalian tumour cells (Waksman *et al.* 1989). All parts of the plant produce the above mentioned pharmaceutically active substance (Dreyer *et al.* 1988). However, the peroxisomicine A_1 concentration is very variable and depends both on environmental conditions (Guerrero *et al.* 1987) and on collection time (Waksman *et al.* 1991). The study of the relationship between primary and secondary metabolism of these species is important for both the determination of optimal growth conditions and optimization of the production of anthracenones. The pathway of anthracenones synthesis, which is the basis for peroxisomicine A_1 synthesis, is through the reducing sugars and shikimic acid synthesis (*e.g.*, Tomko *et al.* 1989, Taiz and Zeiger 1991). In our previous paper (Masarovičová *et al.* 2000), the direct correlation between photosynthesis (as the source of the reducing sugars) and peroxisomicine A_1 concentration (synthesized from the reducing sugars) was confirmed. The aim of this paper was to determine the concentration of starch, reducing sugars, carbon and hydrogen as well as respiration rate in the roots of *K. humboldtiana* and *K. parvifolia* grown under different temperature regimes.

The seeds of *Karwinskia humboldtiana* Zucc. and *Karwinskia parvifolia* Rose were collected from the natural areas in the states Nuevo León and Sinaloa, Mexico, respectively. Plants were grown from the seeds in the greenhouse. Two-year-old plants were transferred to the growth cabinet (*Klimabox 1300*, *Cita*, Slaný, Czech Republic) and cultivated for two months under different temperature regimes: day/night temperature of 35/20 °C (summer temperature regime, SR) and 20/5 °C (winter temperature regime, WR). Temperature regimes were similar to mean air temperatures in summer and winter in natural areas of the species studied (Nuevo León, Mexico). The other conditions in the growth cabinet

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Abbreviations: KH - *Karwinskia humboldtiana*; KP - *Karwinskia parvifolia*; P_N - net photosynthetic rate; R_D - dark respiration rate; SR - summer temperature regime; WR - winter temperature regime.
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were identical for both the SR and WR: photoperiod 16 h, irradiance 200 μmol m^{-2} s^{-1}, day/night relative air humidity 85/50 %. Because of a lack of experimental plants of *K. humboldtiana* during WR the chemical substances were not determined.

The dark respiration rate of roots was determined as CO_2 efflux with an IRGA (*Infralyt 4*, *VEB Junkalor*, Dessau, Germany) using a closed system. A detached whole root system was placed in the thermostabilised chamber and gas exchange rate was measured in relation to the air temperature in the chamber: SR - 35 °C, WR - 20 °C. The measurement and equipment used have been described in detail by Masarovičová (1997).

Root starch concentration was determined in fresh plant material, extracted with 80 % ethylalcohol and centrifuged at 2 000 *g* for 10 min. The precipitate was solubilised in 3.25 cm^3 52 % $HClO_4$, left to stand on ice for 15 min and centrifuged at 3 000 *g* for 15 min. Concentration of starch in supernatant was determined spectrophotometrically (*Specol K20*, *Carl Zeiss*, Jena, Germany) with anthron solution (0.1 g anthron in 50 cm^3 95 % H_2SO_4) at 630 nm. Reducing sugar concentration in the roots was determined in fresh plant material, extracted with 80 °C water for 30 min. Neutral $Pb(CH_3COO)_2$ was added and solution centrifuged at 3 000 *g* for 20 min. For elimination of Pb, the solution of $Na_2(COO)_2$ was added and centrifuged at 3 000 *g* for 15 min. Concentration of reducing sugars in supernatant was determined spectrophotometrically with 80 % phenol and 5 cm^3 96 % H_2SO_4 at 480 nm (Dubois *et al.* 1956). Concentrations of N, C and H in the roots were determined in dried plant material using *Carlo Erba EA 1108* CHNO elemental analyser (Milano, Italy). Analysis of variance was conducted for all the data.

Table 1. Root respiration rate (R_D) [μmol CO_2 kg^{-1} s^{-1}] of the plants grown under summer (SR) and winter temperature regimes (WR). Means ± SE, n = 6; S* - statistically significant difference at P = 0.05, S** - statistically significant difference at P = 0.01.

Species	Regimes	R_D
K. parvifolia	SR	18.49 ± 0.64
	WR	11.31 ± 2.02
K. humboldtiana	SR	8.34 ± 1.30
	WR	3.84 ± 0.57

	KPWR	KHSR
KPSR	S*	S**
KHWR	S*	S*

Root respiration rate, R_D, of *K. parvifolia* was higher than root R_D of *K. humboldtiana* in SR and WR. Higher R_D was found in plants grown under SR than in plants grown under WR in both *Karwinskia* species (Table 1). In previous experiments, root respiration rate of two-year-old plants of *K. humboldtiana* grown in the growth cabinet under temperature 20/5 °C which correspond to WR was quite comparable (0.178 mg CO_2 kg^{-1} s^{-1}) and was higher than R_D of plants grown in the greenhouse with average daily temperature 8 °C (Lux *et al.* 1996). R_D of two-year-old plants of *K. humboldtiana* and *K. parvifolia* grown at two rates of nitrogen supply (Masarovičová *et al.* 2000) ranged from 0.180 to 0.361 mg(CO_2) kg^{-1} s^{-1}. These findings also corresponded to presented results for plants grown under WR (Table 1).

Starch concentration in the roots of *K. parvifolia* was significantly higher in SR than in WR. Interspecific differences in this parameter were not found (Table 2). The concentration of reducing sugars was significantly higher in WR than in SR, in contrast to the starch concentration. In the roots of *K. humboldtiana*, there was observed higher concentration of reducing sugars than in *K. parvifolia* (Table 2). No differences in total N concentration between species and temperature regimes were found. The roots of *K. parvifolia* showed significantly higher C and H concentrations in WR than in SR. The roots of *K. humboldtiana* contained more C and H than the roots of *K. parvifolia* (Table 2).

Study of chemical composition (starch, reducing sugars) presented in this paper follows up recent studies of medicinal plants *Smyrnium perfoliatum* L. (Lux *et al.* 1995) and *Karwinskia* spp. (Masarovičová *et al.* 1998). The variability of the secondary metabolite perixisomicine A_1 concentration (Guerrero *et al.* 1987, Waksman *et al.* 1989, Masarovičová *et al.* 2000) was related to the variability of the C and H concentrations found in both species. Higher concentrations of N, C and H under WR might be caused by lower consumption of substrate for R_D because of lower R_D detected in this temperature regime (Tables 1 and 2). Quantitative data of total N, C and H concentration presented in this paper are in good agreement with previous studies of the trees *Liriodendron tulipifera* and *Quercus alba* (Wullschleger *et al.* 1997). H concentration was determined in 24 species of slow- or fast-growing herbs (Poorter and Bergkotte 1992). Mean value of H concentration found for roots of slow-growing herbs was 57.0 mg g^{-1} (d.m.) what was similar to our findings (Table 2).

It is generally accepted that reducing sugar concentration decreases (as a consequence of decrease of P_N) and starch content increases (as a reserve component) in relation to seasonal changes (from summer to winter) (*e.g.*, in *Fagus sylvatica*, Lux *et al.* 1997). Anthrachinones or anthracenones, including perixiso-micine A_1, are synthesized by way of reducing sugars and sikimic acid (*e.g.* Tomko *et al.* 1989). As the reducing sugars are produced in primary metabolism (photosynthesis) as well as by decomposition of starch, the content of reducing sugars and starch is variable (Table 2). Close relationship between primary and secondary metabolism was confirmed in our previous paper (Masarovičová *et al.* 2000). However, the above mentioned variability could be also asociated with energy cost (reducing sugars and starch as a substrate for respiration) in relation to leaf persistance during the summer and mainly the winter period. Presented interspecific differences of studied parameters can be also related to the regional differences of both *Karwinskia* species. *K. parvifolia* occurred in the lower ranges above sea level and has limited area of extension. *K. humboldtiana* is more adaptable, it grows in various ranges above sea level and has a larger area of extension (Fernandez Nava 1988).

Table 2. The concentrations [mg g^{-1}(d.m.)] of the starch, reducing sugars, nitrogen, carbon and hydrogen in the roots of the plants grown under the summer (SR) and winter temperature regimes (WR). Means ± SE, $n = 6$. NS - not significant difference; S* - statistically significant difference at $P = 0.05$; S** - statistically significant difference at $P = 0.01$.

Species		starch	reducing sugars	nitrogen	carbon	hydrogen
K. parvifolia	SR	112.28 ± 2.79	167.18 ± 15.13	7.72 ± 0.59	386.68 ± 3.99	50.02 ± 1.50
	WR	67.20 ± 1.12	408.43 ± 38.60	14.21 ± 0.64	421.11 ± 0.71	57.68 ± 1.35
K. humboldtiana	SR	115.18 ±2.91	543.30 ± 37,91	8.64 ± 0.26	409.48 ± 1.56	58.13 ± 1.16

	starch	sugars	N	C	H
KPSR - KPWR	S**	S**	S**	S**	S**
KPSR - KHSR	NS	S**	NS	S**	S**

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