ISSN 1021-4437, Russian Journal of Plant Physiology, 2024, Vol. 71:123. © Pleiades Publishing, Ltd., 2024.

RESEARCH PAPERS

Energy and Pro-/Antioxidant Metabolism of *Heracleum sosnowskyi* Manden. Buds during the Winter Dormancy

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Received April 18, 2024; revised May 14, 2024; accepted May 15, 2024

Abstract—Data on changes in energy status, activity of pro-/antioxidant metabolism during overwintering of *Heracleum sosnowskyi* vegetative buds were obtained. The buds of *H. sosnowskyi* are not endodormant and their growth is limited by the decrease of the soil temperature to negative values at the end of November. The optimum temperature for energy storage in autumn was found to be in the range of low positive temperatures (2–5°C). The autumn buds were characterized a high capacity for the cytochrome respiratory pathway (CP, V_{cyt}), which accounted for 78% of the total respiration. During autumn morphogenesis and winter dormancy of buds, the levels of pro-oxidants, which are the content of thiobarbituric acid reactive substances (TBARS) and H_2O_2 content, remained stable. In December, when a stable snow cover and negative soil temperatures were established, the dormant buds showed 2.5 times lower rate of energy storage and activated alternative respiration capacity (AP, V_{alt}), as indicated by a 2 times lower V_{cyt}/V_{alt} ratio. In early spring, compared to winter dormancy, H_2O_2 levels increased 2-fold and antioxidant enzymes activity increased by 27–78% as insolation increased. Spring buds showed an increase in rate of heat production and a decrease in rate of energy storage, which may be due to spring stress caused by increased insolation. It was concluded that in the tissues of *H. sosnowskyi* buds subjected to exogenous dormancy, an energy balance between dormancy and growth processes is achieved at the level of respiratory capacity and pro-/antioxidant metabolism.

Keywords: *Heracleum sosnowskyi* buds, dormancy, energy balance, cytochrome and alternative respiratory pathways, pro-oxidants, antioxidant enzymes

DOI: 10.1134/S1021443724605780

INTRODUCTION

Plants have developed defence mechanisms to adapt to unfavourable environmental conditions, such as winter dormancy, which is a survival strategy in low negative temperatures of seasonal climate. Woody and herbaceous plants undergo morphogenetic transformations in their buds during autumn due to decreasing day length and nutrient outflow from aboveground parts. These transformations include hardening and transitioning to a state of endodormancy (endogenous, organic or deep dormancy) or paradormancy (exogenous or compulsory dormancy) [1].

The study of plant dormancy is typically linked to hormonal balance, as well as the accumulation of cryoprotectors and reserve substances in dormant buds, such as carbohydrates, antifreeze proteins, and unsaturated fatty acids in membrane lipids [2–4]. There are also alterations in respiratory metabolism and the pro-/antioxidant balance [5]. These factors obstruct growth processes and contribute to the redistribution of resources needed to maintain cellular structures.

New data on the molecular mechanisms that regulate the dormancy and regrowth of buds have become available. Studies suggest that the energetically inefficient alternative respiratory pathway (AP), via alternative oxidase (AOX), is involved in maintaining bud dormancy. Dormant axillary bud tissues of rose bush showed a high level of expression of genes encoding alternative oxidase type 2 (AOX2), and accumulation of the corresponding protein [6]. Premature interruption of winter dormancy of blueberry buds decreased in energy storage rate and resulted in an enhanced capacity of AP [7]. AOX, a component of the mitochondrial electron-transport chain, redirects electrons from the ubiquinone pool, directly reducing oxygen to water without contributing to the proton gradient, which severely limits ATP production [8].

The control of ROS metabolism by phytohormones is an important regulator of the maintenance of dormancy and the emergence of plants [9]. Recent studies have shown that the dormancy of rose bush and grapevine buds is linked to a high intracellular hydrogen peroxide (H_2O_2) content [10–12]. Accumu-

lation of H_2O_2 indirectly affects meristem activity by inhibiting the expression of cyclins and cyclin-dependent kinases, leading to arrest of bud growth and cell cycle entry into S phase. The accumulation of H_2O_2 within dormant tissues is thought to be controlled by ABA, with the involvement of antioxidant enzymes. The role of the antioxidant system, comprising high and low molecular weight substances, is crucial in the processes associated with budbreak [10, 13, 14].

Perennial plants have an important adaptation to seasonal climate known as geophilia. Dormant buds of perennial grasses overwinter at a depth in the soil, characterized by high water content and active morphogenesis until late autumn [15]. Sosnowsky's hogweed (*Heracleum sosnowskyi*) is a good model for studying the buds morphogenesis and dormancy. Terminal dormant buds are formed on the top of underground caudexes. The depth of buds in the soil is about 10–20 cm, depending on the plants age [16]. Our observations indicate that the buds of *H. sosnowskyi* have an exogenous dormancy (paradormancy) and their growth is restricted by low temperatures. We suggest that an essential mechanism for the morphogenesis and dormancy of buds is the active control of energy, which involves pro-/antioxidant metabolism.

The objective of this study was to investigate the changes in the energy status, activity of pro-/antioxidant metabolism during the overwintering of *H. sosnowskyi* buds. This investigation is conducted for the adaptive mechanisms of morphogenesis and dormancy of the herbaceous perennial understanding.

MATERIALS AND METHODS

Object of study and conditions for plant cultivation. The study model focused on the terminal buds of *Heracleum sosnowskyi* Manden., which are formed at the top of the underground caudex [16]. Experiments were conducted in 2020–2023 at an experimental plot with *H. sosnowskyi* thickets near Syktyvkar (geographic coordinates are 61.645594° N, 50.731994° E). Soil temperature was measured at a depth of approximately 10 cm using autonomous temperature recorders TR-1 (Engineering Technologies, Russia) every 3 h from September to April. Snow cover height was characterized using data from the hydrometeorological station of Syktyvkar (WMO index 23804), obtained from the website https://rp5.ru/.

During the autumn (September to November), winter (December), and spring (March to April) seasons, we excavated vegetative adult plants, washed them, and selected terminal buds for research purposes. We measured calorespirometric parameters, water freezing point, tissue water content, respiration rate and respiration pathways capacity, pro-/antioxidant metabolism in the bud tissues.

The freezing point of water in the buds was determined using a differential scanning calorimeter (DSC-60, Shimadzu, Japan). Bud segments (apical part) measuring approximately 5 mm in length were placed in an aluminium container with a volume of 100 mm^3. The samples were frozen from 5°C to –30°C at a rate of 1°C/min. The temperature at which water crystallization began (i.e. the beginning of the water phase transition to ice) was calculated using TA-60WS software. The free water was estimated based on the amount of water subjected to phase transition, which was calculated based on the crystallization-heat coefficient for the water–ice phase transition 330 J/g. After measurements, the material was dried at 105°C to a constant dry weight. The water content of the tissue was estimated by calculating the difference between the fresh and dry weight of the samples and expressed as a percentage. The quantitative values of water fractions in tissues were expressed as fractions relative to the total water content [17]. Each determination was performed using five samples of fresh material.

Calorespirometric parameters. Heat production (q, μW/mg dry wt) and respiration (Rco_2, nmol/(mg dry wt s)) were measured by direct calorimetry on a Biotest-2 microcalorimeter (Institute of Biological Instrumentation, Russian Academy of Sciences, Pushchino, Russia). Each cell of the Biotest-2 contained one bud from one plant (fresh weight 150–200 mg). The respiration rate was determined by heat effect of the reaction between CO_2 released by the object and 0.4 M NaOH. The energy storage rate for growth in energy stored equivalents ($\Delta H_B R_{SG}$, μW/mg dry wt) was calculated as the difference between the energy generated by respiration and the amount of heat dissipated. The thermodynamic model [18] was used for this calculation. To investigate the impact of temperature on buds metabolism, we measured heat production, respiration, and energy storage rates at temperatures ranging from 2 to 30°C. We used four to seven fresh material samples for each temperature.

Respiration rate and the respiratory pathways capacity. The respiration rate of buds was determined by measuring O_2 uptake at 20°C using a polarographic Oxytherm system (Hansatech Inst., UK). To prepare the samples, the tops of buds that were 3–5 mm length were cut with a blade and the buds were freed from external cover scales. Cuttings of freshly collected buds fresh weight of 15–20 mg were then placed in a reaction vessel containing HEPES buffer (50 mM, pH 7.2). The samples were kept under constant stirring during the measurement. The respiratory pathways capacity was measured using specific inhibitors method. Optimal concentrations of respiratory inhibitors were selected in preliminary experiments using the direct titration method with increasing concentrations of inhibitors until saturation of O_2 uptake by buds tissues. A 10 mM solution of salicylhydroxamic acid (SHAM) (Lancaster, England) was used as an inhibitor of alternative oxidase (AOX). The activity of cytochrome oxidase was inhibited using a 4 mM KCN (Sigma, USA). The

oxygen uptake rate was calculated as the sum of individual components to determine the individual respiratory pathways capacity:

$$V_t = V_{alt} + V_{cyt} + V_{res},$$

where V_t is total respiration rate, V_{alt} is alternative respiratory pathway capacity inhibited by the alternative oxidase inhibitor SHAM, V_{cyt} is cyanide-sensitive respiratory pathway capacity, and V_{res} is residual respiration recorded in the presence of alternative and cytochrome respiratory pathways inhibitors.

The total respiration rate was determined by measuring the O_2 consumption by tissues without the addition of inhibitors. The capacity of the alternative respiratory pathway was defined as SHAM-sensitive respiration after subtracting residual respiration. The cytochrome respiration capacity was defined as KCN-sensitive respiration in the presence of SHAM after subtracting residual respiration. Residual respiration rate was measured after the addition of SHAM and KCN. After measuring total O_2 uptake rate, respiratory inhibitors were added to the sample sequentially. Measurements were performed in 4–6-fold biological replicates.

Pro-/antioxidant status measuring. The lipid peroxidation activity was determined by the content of thiobarbituric acid reactive substances (TBARS) [19]. The amount of TBARS was calculated using the molar extinction coefficient ($\varepsilon = 156\ mM^{-1}\ cm^{-1}$) after subtracting the nonspecific absorbance at 600 nm.

The H_2O_2 content was assessed using a chemiluminescent method based on the peroxidation of luminol [20]. The amount of H_2O_2 was calculated from the calibration plot. Specificity was tested by inhibiting H_2O_2 formation by adding catalase (Sigma, United States).

The superoxide dismutase (SOD) activity was determined by the ability of the enzyme to suppress the photochemical reduction of nitroblue tetrazolium (NBT) (DDL, United States) [21]. The ascorbate peroxidase (APX) activity was determined by the change in the optical density of the solution as an ascorbate oxidation result [22]. The guaiacol peroxidase (GPX) activity was determined using a method based on the oxidation reaction of guaiacol (Sigma, United States) to tetraguaiacol [23]. The catalase (CAT) activity was measured by the amount of decomposed H_2O_2 (Sigma, United States) per unit time [24]. The soluble protein content was analyzed according to Bradford [25]. Protein isolation procedures were carried out at 4°C.

Determining the activity of antioxidant enzyme isoforms. To determine the isoenzyme composition of SOD and CAT, the method of native electrophoresis in polyacrylamide gel (12.5% for SOD, 10% for CAT) was used. Native phoresis was carried out at 4°C and at a stable current of 180 V, as described in [26]. From 10 to 20 μg of protein was added to the sample wells at the top of the gel.

To identify SOD isoforms, gels were incubated in phosphate buffer with the addition of riboflavin and NBT in the dark [21]. To inhibit Cu/Zn-SOD and Fe-SOD, 5 mM H_2O_2 was added to the phosphate buffer. Cu/Zn-SOD selective inhibition occurred when the gels were incubated in a buffer containing 3 mM KCN. CAT isoforms visualization was determined by a reduction of potassium hexacyanoferrate (III) to potassium hexacyanoferrate (II) and the subsequent reaction of potassium hexacyanoferrate (II) with iron (III) chloride to form a colored compound [27]. To process images of protein profiles of antioxidant enzyme isoforms on gels, the GelDoc-ChemiDocXRS gel documentation system (Bio-Rad, United States) and Quantity One Analysis Software, Version 4.6.9 (Bio-Rad, United States) were used. The calculations used the average pixel density of the lanes minus the pixel density values of the gel background.

Statistical analysis. The data was statistically treated using the Statistica 10 software (StatSoft Inc., United States). Normality of distribution was estimated using the Shapiro-Wilk's test. The means were compared using one-way ANOVA (Duncan test). All calculations were performed at a significance level of $P \leq 0.05$. The tables and figures show the means and their standard errors.

RESULTS

Soil Temperature Regime at the Depth of Bud Occurrence

Significant fluctuations in soil temperature were observed from September to April (Table 1). In September, the soil temperature was relatively high, reaching 10°C. By the end of November, the temperature had decreased to negative values, and permanent snow cover was observed in early December. Throughout the winter months and the first month of spring, soil temperatures remained stable at around –0.5 to –1°C, and snow cover increased from 20 to 50 cm. In the third decade of April, with active snow melting, the soil temperature increased to positive values.

Calorimetric Parameters and Energy Status of Buds

The measurements of water freezing temperature in tissues of *H. sosnowskyi* buds showed stable values during the plant's overwintering (Table 2). In late April, when the soil temperature at the depth of the buds habitat was positive, the water freezing temperature in tissues increased by an average of 1.5 degrees compared to the autumn, winter and early spring. The buds tissues were found to have a high water content (83–93%) throughout the annual development cycle. Additionally, the tissues contained a relatively high proportion of frozen (free) water, approximately 80%.

Calorimetric calculation showed a linear increase in heat production from 4 to 34 μW/mg dry wt in the

Table 1. Soil temperature at the depth of *Heracleum sosnowskyi* buds and snow cover depth

Month	Decade of the month	Temperature, °C	Snow depth, cm
September	I	+11.8	–
	II	+9.9	
	III	+10.5	
November	I	+1.0	6*
	II	+1.2	
	III	–0.4	
December	I	–0.6	21
	II	–0.8	
	III	–0.6	
January	I	–0.6	38
	II	–0.6	
	III	–0.5	
February	I	–0.5	47
	II	–0.5	
	III	–0.6	
March	I	–0.5	51
	II	–0.5	
	III	–0.5	
April	I	–0.3	17*
	II	–0.3	
	III	+2.5	

*—non-permanent snow cover.

temperature range of 5–30°C (Table 3). Similarly, the respiration rate in this temperature range exhibited growth dependence, although the differences were less pronounced. The results of the calculations indicate that the respiration efficiency, which refers to the storage of energy formed during respiration, was highest at a low positive temperature of 2°C for autumn buds. However, it decreased by 2–4 times at moderate and high temperatures. Additionally, the value of the $q/455Rco_2$ ratio was lowest at 2–5°C and increased by 3–4 times with increasing temperature.

During the overwintering period, from September to December, we observed significant dynamics of metabolic heat production and energy storage rate in bud tissues of *H. sosnowskyi*, measured calorimetrically (Table 4). The heat production remained stable during the autumn but increased almost two-fold in December. The respiration rate remained relatively constant over the whole period of autumn and winter. The calculations indicate that the amount of stored energy, which was relatively high in early autumn (September), decreased by 2.5 times in November, when the soil temperature at the depth of buds dropped to –1°C.

During the spring season, specifically from March to mid-April, the rate of heat production more than doubled, and the rate of CO_2 release increased threefold (Table 4). As a result, by mid-April, the bud tissues stored energy value was about 28 μW/mg dry wt, which was 4.5 times higher than in winter and early spring. In late April, when the soil temperature became positive, the rate of heat production continued to increase and reached maximal values, approximately 37 μW/mg dry wt. During this period, the respiration rate remained constant, while the energy storage rate in the buds was reduced by a three-fold.

Respiration Rate and Respiratory Pathways Capacity in the Buds

The O_2 uptake rate in *H. sosnowskyi* buds ranged from 3750 to 5440 nmol O_2/(g dry wt min) (Table 5). The capacity of the energy-efficient cytochrome pathway (V_{cyt}) dominated in bud O_2 consumption, contributing to 60–80% of total respiration (Fig. 1).

During the preparation of *H. sosnowskyi* for overwintering in September, when the temperature at the depth of buds was +10°C, meristematic tissues exhibited a high V_{cyt} capacity and a low capacity of the alternative respiratory pathway (V_{alt}) (Table 5). At this

Table 2. Water content and freezing point in *Heracleum sosnowskyi* buds

Month	Water freezing point, °C	Water content, %	Share of frozen water, %
IX	-8.0 ± 0.4^{a}	88.0 ± 1.3^{a}	81.2 ± 4.6^{ab}
XI	-8.9 ± 1.0^{a}	82.7 ± 1.2^{ab}	82.5 ± 5.2^{a}
XII	-9.2 ± 0.9^{a}	83.2 ± 2.5^{a}	75.7 ± 6.2^{a}
III	-8.3 ± 0.8^{a}	86.3 ± 1.4^{ab}	81.9 ± 6.7^{a}
IV	-7.3 ± 0.3^{ab}	87.5 ± 3.5^{a}	98.3 ± 5.0^{b}
IV*	-5.8 ± 0.8^{b}	92.9 ± 1.2^{ab}	78.4 ± 7.5^{ab}

Mean values and their standard errors are presented. Different superscripted letters indicate statistical significance of the parameters differences depending on the month (ANOVA, Duncan test, $P \le 0.05$, $n = 5$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid- and late April, respectively.

Table 3. Dependence of *Heracleum sosnowskyi* buds calorimetric parameters on temperature in the autumn period

Temperature, °C	q, μW/mg dry wt	Rco_2, nmol/(mg dry wt s)	$\Delta H_B R_{SG}$, μW/mg dry wt	$q/455Rco_2$
2	5.7 ± 1.2	0.06 ± 0.01	21.1 ± 3.5	0.2 ± 0.03
5	3.8 ± 0.6	0.03 ± 0.003	11.1 ± 1.1	0.2 ± 0.03
10	10.2 ± 1.4	0.04 ± 0.003	9.6 ± 2.2	0.5 ± 0.06
15	15.3 ± 1.2	0.05 ± 0.006	8.2 ±1.5	0.6 ± 0.03
20	25.4 ± 1.2	0.07 ± 0.004	7.5 ± 1.6	0.8 ± 0.05
25	32.0 ± 2.9	0.08 ± 0.005	5.2 ± 1.4	0.8 ± 0.06
30	34.0 ± 1.4	0.09 ± 0.006	9.5 ± 3.4	0.8 ± 0.07

q, heat production rate; Rco_2, respiration rate; $\Delta H_B R_{SG}$, energy storage rate; $q/455Rco_2$ ratio, metabolic efficiency index. Mean values and their standard errors are presented.

Table 4. Metabolic heat production, respiration and energy storage rate in *Heracleum sosnowskyi* buds

Month	q, μW/mg dry wt	Rco_2, nmol/(mg dry wt s)	$\Delta H_B R_{SG}$, μW/mg dry wt	$q/455Rco_2$
IX	13.8 ± 0.9^{a}	0.06 ± 0.004^{ab}	13.6 ± 1.4^{b}	0.5 ± 0.03^{c}
XI	16.2 ± 2.1^{a}	0.05 ± 0.01^{ab}	5.6 ± 1.2^{a}	0.8 ± 0.04^{b}
XII	27.6 ± 1.7^{b}	0.07 ± 0.01^{b}	5.3 ± 2.0^{a}	0.9 ± 0.04^{a}
III	13.2 ± 2.5^{a}	0.04 ± 0.01^{a}	5.9 ± 2.5^{a}	0.7 ± 0.1^{a}
IV	29.0 ± 5.6^{b}	0.12 ± 0.02^{c}	27.5 ± 3.9^{b}	0.5 ± 0.07^{a}
IV*	37.4 ± 4.3^{b}	0.102 ± 0.01^{c}	9.1 ± 1.1^{b}	0.8 ± 0.05^{b}

q, heat production rate; Rco_2, respiration rate; $\Delta H_B R_{SG}$, energy storage rate; $q/455Rco_2$ ratio, metabolic efficiency index (if greater than one, growth stops). Mean values and their standard errors are presented. Different superscripted letters indicate statistical significance of the parameters differences depending on the month (ANOVA, Duncan test, $P \le 0.05$, $n = 5–7$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid- and late April, respectively.

Table 5. Respiration rate and the respiratory pathways capacity in *Heracleum sosnowskyi* buds

Month	V_t	V_{cyt}	V_{alt}	V_{res}	V_{cyt}/V_{alt}	EER ($Y_{ATP/glucose}$)
IX	4147 ± 141^{a}	3244 ± 143^{ac}	632 ± 87^{b}	271 ± 49^{a}	5.7 ± 0.9^{b}	26 ± 0.4^{b}
XI	3744 ± 87^{a}	2272 ± 229^{b}	1053 ± 261^{ab}	420 ± 70^{ab}	2.80 ± 0.7^{a}	23 ± 1.2^{a}
XII	4287 ± 204^{a}	2790 ± 86^{ab}	1130 ± 133^{ab}	367 ± 53^{ab}	2.64 ± 0.3^{a}	24 ± 0.4^{ab}
III	5440 ± 195^{b}	3771 ± 321^{c}	1289 ± 245^{a}	380 ± 41^{ab}	3.61 ± 1.1^{ab}	24 ± 0.8^{ab}
IV	5266 ± 354^{b}	3270 ± 252^{ac}	1480 ± 237^{a}	516 ± 31^{b}	2.42 ± 0.5^{a}	23 ± 0.7^{a}
IV*	4346 ± 260^{a}	2774 ± 152^{ab}	1192 ± 188^{ab}	380 ± 44^{ab}	2.90 ± 0.8^{a}	24 ± 0.7^{a}

Mean values and their standard errors of the total respiration rate (V_t), cytochrome (V_{cyt}) and alternate (V_{alt}) respiratory pathways capacity and residual respiration rate (V_{res}) (nmol O_2/(g dry wt min)) are presented, cytochrome and alternative respiratory pathways capacity ratio (V_{cyt}/V_{alt}) and the energy efficiency of respiration (EER) calculated from the value of the $Y_{ATP/glucose}$ ratio, which characterizes the theoretically possible amount of ATP formed in the mitochondrial ETC during the oxidation of 1 mole of glucose [31]. Different superscripted letters indicate statistical significance of the parameters differences depending on the month (ANOVA, Duncan test, $P \le 0.05$, $n = 4–6$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid- and late April, respectively.

stage, CP contributed to 78% of the buds total O_2 uptake while AP accounted for no more than 16% of total respiration (Fig. 1).

In November, significant changes in the capacity and ratio of respiratory pathways were observed while maintaining a high total respiration rate. The V_{cyt} capacity decreased significantly, while there was a tendency to increase the V_{alt} capacity. In December, the trend of lower V_{cyt} and higher V_{alt} capacities persisted compared to September (Table 5). The V_{cyt}/V_{alt} ratio

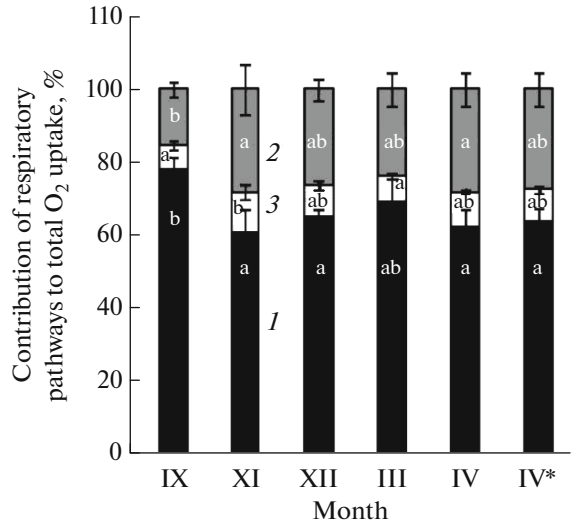

Fig. 1. Relative contribution of cytochrome (*1*), alternative (*2),* and residual (*3*) respiration to the total O_2 uptake by *Heracleum sosnowsky* buds in different months. Different letters on the columns indicate the statistical significance of the parameter differences depending on the month (ANOVA, Duncan test, $P \le 0.05$, $n = 4–6$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid and late April, respectively.

during this period was approximately 3, which is half of what it was in the early autumn period.

In March and mid-April, respiratory activity of buds increased by 1.3 times compared to the winter period and reached its maximum value. The V_{cyt}/V_{alt} ratio also increased by almost 1.5 times in March, but decreased again by April and averaged at 2.5. Towards the end of April, when temperatures rose above zero, the total O_2 uptake rate decreased by 20% from its maximum values due to a more significant decrease in V_{cyt}.

The residual respiration, which is not related to the activity of mitochondrial ETC enzymes, varied between 270 and 500 nmol O_2 depending on the season. Its proportion did not exceed 11% of the buds total respiratory activity.

Pro-/Antioxidant Status of the Buds

The TBARS and H_2O_2 content remained relatively stable from September to March in *H. sosnowskyi* buds (Table 6). Significant changes in the content of prooxidants were observed in the middle and end of April. The amount of TBARS decreased by more than 1.4–1.8 times, and H_2O_2 content doubled.

In September and November, the antioxidant enzymes activity was minimal. In December, when the soil temperature decreased to −1°C, there was a significant increase in the SOD and GPX total activity (Table 6). The maximum activity of all investigated enzymes in the buds was observed in early spring. During this period, preceding bud growth, the level of enzyme activity increased by 27–78% compared to December. The level of catalase activity increased significantly by the end of April.

The Mn- and Cu/Zn-containing SOD isoforms were identified in the buds (Fig. 2). The Mn-containing isoform contributed the most to the total SOD activity. The changes in the activity of SOD isoforms were comparable to the changes in enzyme total activity.

In autumn and winter, CAT was represented by one isoform. However, in the spring, it was represented by two isoforms (Fig. 2). The activity of the first isoform remained stable throughout the study period. The increase in total catalase activity in the spring is due to the new isoform appearance.

DISCUSSION

The terminal overwintering buds of *H. sosnowskyi* are forming at the underground caudex top. The caudex is buried 10–20 cm deep in the soil [16], which freezes to −1°C at this depth during winter (Table 1). *H. sosnowskyi* bud tissues' water content remained

Table 6. Pro-/antioxidant metabolism parameters of *Heracleum sosnowskyi* buds

Month	TBARS, μmol/g of dry wt	H_2O_2, μmol/g of dry wt	SOD, units/mg protein	APX, μmol ascorbate/ (mg protein min)	GPX, μmol guaiacol/ (mg protein min)	CAT, μmol H_2O_2/ (mg protein min)
IX	9.4 ± 0.3^{ab}	7.7 ± 0.5^{a}	2.3 ± 0.1^{a}	1.0 ± 0.1^{a}	0.20 ± 0.2^{a}	19.4 ± 0.7^{ab}
XI	10.0 ± 0.9^{ab}	6.7 ± 0.2^{a}	2.1 ± 0.2^{a}	0.9 ± 0.2^{a}	0.16 ± 0.03^{a}	17.6 ± 2.4^{a}
XII	9.6 ± 1.0^{ab}	7.1 ± 0.2^{a}	3.5 ± 0.1^{b}	0.9 ± 0.2^{a}	0.38 ± 0.02^{b}	19.0 ± 0.1^{ab}
III	10.7 ± 1.0^{b}	9.9 ± 1.9^{a}	3.7 ± 0.2^{b}	1.5 ± 0.2^{b}	0.56 ± 0.04^{c}	20.4 ± 1.5^{adc}
IV	8.2 ± 0.7^{a}	17.7 ± 1.9^{b}	4.5 ± 0.2^{c}	1.8 ± 0.2^{b}	0.59 ± 0.06^{c}	21.8 ± 2.4^{bc}
IV*	5.4 ± 0.6^{c}	17.1 ± 0.9^{b}	4.5 ± 0.4^{c}	1.6 ± 0.2^{b}	0.57 ± 0.18^{c}	24.2 ± 0.8^{c}

TBARS, the content of lipid peroxidation substances; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; CAT, catalase. Mean values and their standard errors are presented. Different superscripted letters indicate statistical significance of the parameters differences depending on the month (ANOVA, Duncan test, $P \le 0.05$, $n = 4–9$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid- and late April, respectively.

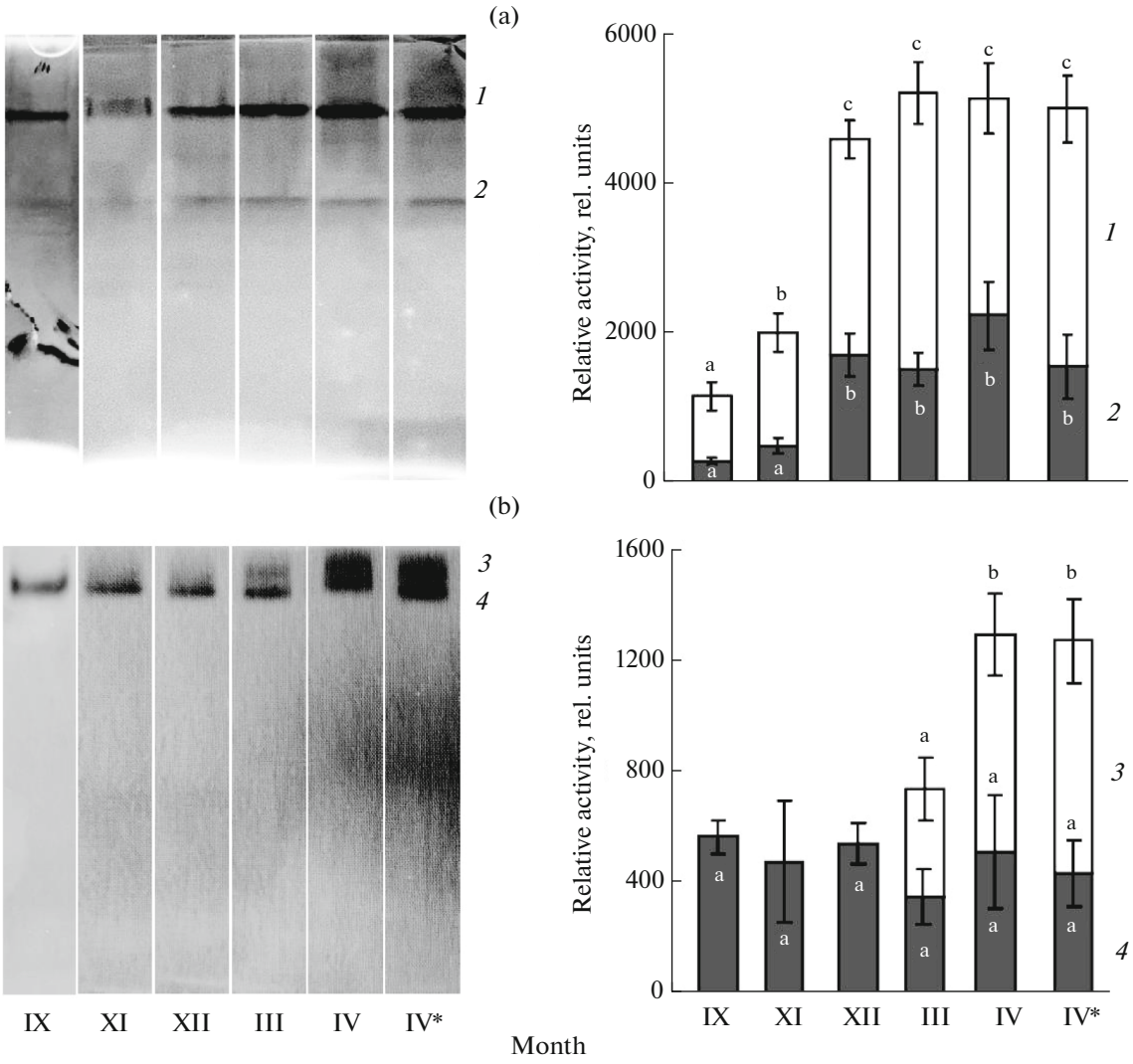

Fig. 2. Protein profiles of superoxide dismutase (SOD) isoforms (a) and catalase (CAT) (b) (left) and their relative activities (right) in *Heracleum sosnowskyi* buds. (*1*) Mn-containing isoform for SOD, (*2*) Cu/Zn-containing isoform for SOD, (*3, 4*) different isoforms of CAT. Mean values of the isoforms relative activity and their standard errors are presented. Different letters on the columns indicate the statistical significance of differences between the individual enzyme isoforms activities depending on the month (ANOVA, Duncan test, $n = 4–9$, $P \le 0.05$). IX, XI, XII, III, IV, IV*—September, November, December, March, mid- and late April, respectively.

consistently high, with a 76–98% of free water, throughout the study period. The freezing temperature of water in the buds ranged from –6 to –9°C (Table 2). In contrast, axillary buds of woody plants [28] and shrubs [7] typically have lower water content, around 50–55%, and can withstand water freezing temperatures as low as –12°C in winter. Thus, the diving deep into the soil of *H. sosnowskyi* buds is an important adaptation to seasonal climate. It contributes to the plant's protection from freezing temperatures and allows it to avoid dormant. Experiments have shown that 30–60% of adult *H. sosnowskyi* plants retain the viability of buds after exposure to temperatures of minus 4–5°C [29].

It was found that the energy storage efficiency ($\Delta H_B R_{SG}$) formed in the respiration of *H. sosnowskyi* buds is relatively high at low positive temperatures during the autumn (Table 3). Based on the $\Delta H_B R_{SG}$ and $q/455Rco_2$ ratio values, temperatures ranging from 2–5°C are optimal for growth and efficient energy use in the morphogenetic processes of the *H. sosnowskyi* buds in autumn. Similar results were obtained for the adaptation of underground buds during preparation for overwintering in rhizomes of *Phalaroides arundinacea* and *Achillea millefolium*, which continued to grow until late autumn [15, 30].

The high V_{cyt} capacity, which accounted for 78% of the total respiration, and the high $Y_{ATP/glucose}$ ratio values (Table 5) provide evidence of the relatively high energetically metabolism level of *H. sosnowskyi* buds in early autumn. Amthor [31] calculated that the ATP

yield during the complete oxidation of a glucose mole ($Y_{ATP/glucose}$) is 29 moles when the main (cytochrome) respiratory pathway is fully loaded, and 11 moles during respiration by the alternative respiratory pathway. The $Y_{ATP/glucose}$ value, which takes into account the involvement of AP in the respiration of buds in the autumn, was calculated to be 26. The $Y_{ATP/glucose}$ values are typical of cereal leaves during their active growth period [32]. In mature leaves of *Plantago media* during the flowering phase of plants, the $Y_{ATP/glucose}$ value did not exceed 20 [33]. The data obtained indicate a high energy status of *H. sosnowskyi* buds during early autumn.

During the late autumn period (late November), when the soil temperature decreased to negative values (Table 1), there was a significant decrease in the amount of stored energy in the *H. sosnowsky* bud tissues. This low level was maintained throughout the winter (Table 4). At the same period, a decrease in V_{cyt} respiration was observed, while the energetically low-efficient V_{alt} contribution to the total respiration increased, accounting for 27% of total respiration (Fig. 1). The AP activation in the buds may indicate the participation of AOX in maintaining the energy balance of meristematic tissue cells during overwintering when exposed to low temperatures. It was shown a significant increase in the expression of genes encoding second type alternative oxidase (AOX2) and accumulation of the corresponding protein in the tissues of axillary buds of dormant rose bushes [6]. AOX redirects electrons from the ubiquinone pool to O_2, bypassing the cytochrome pathway complexes in the mitochondrial respiratory chain. This may reduce ATP formation and lead to a low-energy status that inhibits growth. During the buds overwintering, the respiration energy efficiency coefficient values in bud tissues remained consistently high (Table 5). This suggests that there is a energy balance between the dormant and growth processes in the meristematic tissues of *H. sosnowskyi* buds with paradormancy (exogenous dormancy), which can achieved through respiratory pathways activity regulation.

In the buds of *H. sosnowsky*, the TBARS and H_2O_2 content remained unchanged during the winter compared to the autumn period (Table 6). This could be attributed to the activity of antioxidant enzymes that neutralize ROS. As the soil temperature decreased to negative in November-December, there was a significant increase in SOD and GPX activity, followed by the APX activation. High molecular weight antioxidants are crucial in maintaining the optimal ROS level during the woody plants buds dormancy [10, 11]. SOD is involved in the superoxide anion radical dismutation to H_2O_2. Increased peroxidase activity aims to control the H_2O_2 content in dormant buds. Catalase has been found to have a lower affinity for H_2O_2 compared to peroxidases, which may explain its stable level of activity during this period. Throughout the morphogenesis of *H. sosnowsky* buds, the Mn-containing SOD isoform (Fig. 2) contributed the major share of the total SOD activity. It is well known that Mn-SOD is localized in mitochondria. Its high activity may be associated with the relatively high respiration rate of buds during overwintering (Table 4).

In spring, during mid-April, there was a significant increase in the energy storage rate due to the melting of snow cover and an increase in light resources at the soil level (Table 4). An increase in the total respiratory activity of buds was observed in March and April (Table 5) due to the enhanced capacity of CP. At the same time the AP capacity also tended to increase. In April, a relatively high level of H_2O_2 and low lipid peroxidation activity were found (Table 6). The maximum activity of antioxidant enzymes was observed in this case. SOD activity increased primarily due to the involvement of Mn-SOD, while CAT activity increased due to the appearance of an additional isoform. Previous studies [10, 14] have demonstrated that the emergence of buds in rose and raspberry bushes from dormancy is linked to an increase in the activity of the antioxidant system. The obtained results suggest that the rearrangement of bud metabolism is associated with the transition of plants to growth and development, which is influenced by adaptation to increased insolation levels. Measurements showed that in April, after snow melt, 680 μmol/m^2 s of PAR was delivered to the soil surface in the studied area, whereas in March, under snow cover, only 6 μmol/m^2 s of PAR was observed. In a previous study, we demonstrated the role of the pro-/antioxidant system and the involvement of AP in *Achillea millefolium* rhizomes greening tops photoprotection during the photomorphogenetic transition from underground to aboveground growth [34]. During the photophilic stage of morphogenesis of underground shoots, there was an increase in TBARS and H_2O_2 content, and antioxidant enzymes activity. H_2O_2 and other components of pro-/antioxidant metabolism can serve as signalling molecules, modulating plant growth and development, and participating in the regulation of organogenesis. They help maintain the balance between cell proliferation and differentiation in apical meristems [9, 35, 36].

CONCLUSIONS

The study of morphogenesis and dormancy physiological mechanisms of *H. sosnowskyi* buds revealed their functional adaptations during plant overwintering. The buds do not have endodormancy and their growth is limited by a decrease in soil temperature to negative values at the beginning of winter. The optimal temperature for energy storage during the autumn period was found to be in the range of low positive temperatures (2–5°C). This suggests that the morphogenetic processes of buds are adapted to wintering

conditions. Autumn buds were characterized by a high capacity of the cytochrome respiratory pathway, which accounted for 78% of the total respiration. During autumn morphogenesis and winter dormancy of buds, the level of pro-oxidants, which are TBARS and H_2O_2, remained stable.

In December, when stable snow cover is established and the soil temperature drops, the dormant buds are characterized by a 2.5-fold decrease in stored energy and a 2-fold decrease in the V_{cyt}/V_{alt} ratio. The activation of alternative respiration capacity in bud tissues indicate the involvement of alternative oxidase in maintaining the energy balance of meristematic tissues and regulating dormant of buds. This is associated with a decrease in the energy efficiency of respiration and growth delay.

In spring, during April, there was a twofold increase in H_2O_2 content and a 30–100% increase in all antioxidant enzymes compared to winter, as insolation at soil level increased. The metabolism of H_2O_2 may be involved in the adaptation of buds to growth in the light and play an important role in triggering gene expression of antioxidant enzymes and AOX for photoprotection. Currently, enhancing the capacity of both CP and AP has been shown to increase total respiratory activity. Spring buds are characterized by an increased the heat production and a threefold decrease in the amount of stored energy. This may be the result of spring stress from increased insolation. It was concluded that an energy balance is achieved between the dormancy and morphogenetic in the tissues of *H. sosnowskyi* buds with paradormant, in terms of respiratory pathways capacity and pro-/antioxidant metabolism during overwintering.

ABBREVIATIONS AND NOTATION

CP	cytochrome respiratory pathway
AP	alternative respiratory pathway
AOX	alternative oxidase
V_t	total respiration
V_{alt}	capacity of alternative respiratory pathway
V_{cyt}	capacity of cytochrome respiratory pathway
V_{res}	residual respiration
EER	energy efficiency of respiration
TBARS	thiobarbituric acid reactive substances
H_2O_2	hydrogen peroxide
SOD	superoxide dismutase
APX	ascorbate peroxidase
GPX	guaiacol peroxidase
CAT	catalase

FUNDING

The work was carried out within the framework of the theme of state-budget R&D "Photosynthesis, respiration and bioenergetics of plants and phototrophic organisms (physiological-biochemical, molecular-genetic and ecological aspects)" (registration number 1021062311434-4-1.6.11;1.6.19).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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