RESEARCH ARTICLE

**UPBEAT1-ROS-POD-PAL system under different xylogenesis’ scenarios in Karelian birch (Betula pendula Roth var. carelica (Mercl.) Hämet-Ahti).**


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**Abstract:**

**Background:** We studied **UPBEAT1** (*UPB1*) which regulates superoxide radical / hydrogen peroxide ratio together with peroxidase (POD) activity and **PAL** genes expression under different ways of apical meristem development during the xylem structural elements’ formation in unique woody plants *B. pendula* var. *pendula* with straight-grained wood and *B. pendula* var. *carelica* with figured wood. The differentiation process predominances in straight-grained wood (**B. pendula** var. *pendula*) or proliferation – in the figured wood. The investigation was conducted in the radial row (cambial zone - differentiating xylem - mature xylem) during the active cambial growth period. **Objective:** 16-year-old straight-grained silver birch (*Betula pendula* Roth) and figured Karelian birch (*Betula pendula* Roth var. *carelica* (Mercl.) Hämet-Ahti) with figured wood. **Method:** Hydrogen peroxide and superoxide radical contents, peroxidase activity were determined spectrophotometrically. Gene expression for **PAL** family genes and the **UPBEAT1** gene was performed using qRT-PCR. **Results:** Principal component analysis confirmed that trees with straight-grained and figured wood were different according **UPBEAT1-ROS-POD-PAL** system functioning. **Conclusion:** Higher superoxide radical / hydrogen peroxide ratio in figured Karelian birch, along with **UPBEAT1** transcription factor and **PAL** genes upregulation, distinguished it from straight-grained silver birch. This metabolic picture confirmed the shift of Karelian birch xylogenesis towards proliferation processes, that was accompanied by ROS and phenolic compounds flows involving also POD activity in the coordinated work.
Keywords: superoxide radical; hydrogen peroxide; UPBEAT1; xylogenesis; figured wood; cambium
1. INTRODUCTION

Over the past decade, there has been an emergence of literature focusing on the investigation of UPBEAT1 (UPB1), a transcription factor belonging to the basic/helix-loop-helix (bHLH) family. This factor plays a crucial role in regulating ROS distribution and maintaining the balance between cell proliferation and differentiation in herbaceous plants. It has been observed to modulate the peroxidases (PODs) expression, which plays a crucial role in the antioxidant system (AOS) enzymes. Subsequently, the PODs, which are engaged in AOS activity, control the concentrations of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide radical. The expression of regulators responsible for apical meristem development is modulated by the balance of the ROS studied, which function as signaling molecules [1, 2].

The activity of the lateral meristem, known as the cambium, is driven by the interplay between cell proliferation and differentiation, which influences xylogenesis [3, 4]. The significant role of POD activity in xylogenesis is acknowledged. It has been demonstrated in Arabidopsis plants that there are 500 distinct cell wall proteins categorized into 9 functional classes, with a significant portion (14.6%) being oxidoreductases, predominantly AOS enzymes, and nearly half of them falling under POD [5]. AOS enzymes play a crucial role in both cell walls’ formation and cross-linking [6, 7]. Among the PODs that are crucial and highly active, one stands out - the apoplastic POD or cell wall POD. The apoplastic peroxidase activity is identified as a primary contributor to the reactive oxygen species generation [8]. Because of the POD and other enzymes’ activity, reactive oxygen species (ROS) are produced within the cell wall, with hydrogen peroxide being the predominant compound [9].

AOS enzymes, hydrogen peroxide, along with other ROS, play a role in the cell wall lignification process, which relies on monolignol polymerization. The intensification of this process may show a shift toward xylogenesis direction. The constitution of the cellular membranes will be contingent upon the level of AOS activity within the cellular walls, specifically directed towards lignification. The most significant contribution in the redistribution of these processes is attributed to the apoplastic peroxidases of cell walls, as they actively oxidize monolignols in the apoplastic space [10, 11].

Through the reaction of superoxide dismutase, the hydrogen peroxide necessary for POD’s reaction is produced. Superoxide dismutase triggers the reaction to neutralize the superoxide radical to form hydrogen peroxide [12]. The localization of the produced hydrogen peroxide, perhaps more so than its absolute concentration,
could be accountable for the initiation of subsequent reactions aimed at either neutralizing the resultant hydrogen peroxide through catalase (CAT) and/or POD [13]. To support cell division and the proper functioning of cell walls, controlled levels of hydrogen peroxide is crucial [14]. Therefore, the delicate balance between hydrogen peroxide and superoxide radical can have a determining influence, notably in the construction of cell walls with diverse compositions. Moreover, it is imperative to consider the balance between the activities of SOD/CAT/POD enzymes responsible for both generating and consuming these ROS.

The activity of phenylalanine ammonia lyase (PAL) plays a crucial role in the studied processes. PAL is a key enzyme in secondary metabolism that regulates the synthesis of flavonoids, a precursor of lignin, and the expression of the coding gene, PAL [15]. Hydrogen peroxide and superoxide radical serve as substrates during the radicalization of flavonoids in the lignin formation [16]. In addition, phenolic compounds are necessary for POD and polyphenoloxidase (PPO) reactions.

The Karelian birch (Betula pendula Roth var. carelica (Mercl.) Hämet-Ahti) serves as a distinctive model organism for conducting the study of these processes in nature. It is a form of silver birch (Betula pendula Roth). In Karelian birch wood, deviations are observed in the ratio and spatial orientation of its structural elements. Differences can be observed in the timing of origin and patterns of figured wood development in ontogenesis in Karelian birch trees. The unique characteristics of Karelian birch make it an ideal subject for studying xylogenesis mechanisms. It allows for the examination of both "normal and abnormal" scenarios of conducting tissues growth and development within a single trunk, effectively mitigating the influence of environmental factors [4, 17].

Previously, we showed on model objects: straight-grained silver birch (Betula pendula Roth) and figured Karelian birch (Betula pendula Roth var. carelica (Mercl.) Hämet-Ahti) that figured wood is accompanied by an increase in SOD, CAT, POD, and PPO activity during active cambial growth. A significant correlation was discovered among the enzymes that were examined, indicating a complex series of reactions among the studied AOS enzymes during the changing xylogenesis scenario in Karelian birch, ultimately resulting in the development of figured wood [17, 18]. This operation of AOS enzymes involves ROS, especially superoxide radical and hydrogen peroxide. Probably, the increase in the activity AOS enzymes should be a consequence of an increase in ROS content.

Considering all the above, the purpose of this study was to study the expression of the genes of PAL family, encoding phenylalanine ammonia lyase, and UPBEAT1 gene, encoding a transcription factor of the bHLH family, regulating ROS distribution - hydrogen peroxide and superoxide radical, simultaneously with POD activity in the xylem structural elements’ formation during the predominance of
differentiation processes while the formation of straight-grained wood or proliferation during the formation of figured wood in the radial row [19] cambial zone - differentiating xylem - mature xylem during the period of active cambial growth.

2. MATERIALS AND METHODS

2.1 Plant material and sampling

16-year-old *B. pendula* var. *pendula* and *B. pendula* var. *carelica* were planted on the biological station of the Karelian Research Centre of the Russian Academy of Sciences. The seeds used to grow the trees were obtained through controlled pollination conducted by Forelia OY in Finland. The chosen samples of Karelian birch exhibit a significant intensity of figured wood formation. The collected samples were from common silver birches (p) and figured wood birches with figure signs (F) (Figure 1). There were five trees in each group.

At a height of 1.5 m above ground level, plant samples were gathered on 12 July during the cambial growth period. In order to conduct biochemical analysis, 10 cm × 10 cm portions of the trunk were chosen and the bark was detached. As cambial growth occurred, the bark shifted away from the wood in the expanding xylem area. From the inner bark, tissue complexes comprising non-conductive phloem cells, fully formed early phloem, differentiating late phloem, and cambial zone (F1) were collected [19]. To obtain the mature xylem (MX), the tissue layers containing differentiating early xylem cells (F2) were scraped off the wood surface using a blade, as shown in Figure 2. Freezing in liquid nitrogen was done for all tissues, followed by storage at -80°C.

**Figure 1.** Debarked surface common silver birches (*B. pendula* var. *pendula* straight-grained trees) (p), figured birches (*B. pendula* var. *carelica* trees) (F) and tissue complexes, included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone (F1); the layers of tissue, including differentiating early xylem cells (F2), and cells of the mature xylem (MX).

2.2 Biochemical research

To determine enzyme activity, plant tissues were ground with liquid nitrogen and extraction buffer (Hepes 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM EGTA, 3 mM DTT, 5 mM MgCl2, 0.5 mM PMSF) in a tissue: buffer ratio of 1:10. After 15 minutes of extraction at 4 °C, the homogenate was centrifuged at 12,000 g for 10 minutes (Centrifuge MPW-351R, Poland). The supernatant was purified on 20 cm³ columns filled with Sephadex G-250 gel. Next, aliquots containing the largest amount of enzyme protein were collected. In tissues, the protein concentration was 10-50 μg/ml. Protein content was determined using the Bradford method. Enzyme activity was determined spectrophotometrically on a Spectro Star Nano plate spectrophotometer (BMG Labtech, Germany). To determine the activity of peroxidase (POD), guaiacol was used as a hydrogen donor, and hydrogen peroxide was used as a substrate.
The incubation medium for determining POD activity contained 50 mM K, Na-phosphate buffer (pH 5), 2.6 mM hydrogen peroxide, and 21.5 mM guaiacol. Incubation time is 30 minutes. POD activity was determined by the rate of formation of the reaction product, tetraguaiacol (TG). To determine the content of the formed TG, the increase in optical density at 470 nm was measured, and the amount of TG was calculated taking into account the extinction coefficient ($\varepsilon_{470nm} = 0.0266 \mu M^{-1} cm^{-1}$). POD activity was expressed as $\mu$mol of TG formed per 1 mg of protein ($\mu$mol TG/mg protein) [17, 18, 20].

The content of superoxide radical was determined in material frozen at -80°C using a solution of 0.05 mM Azure I and 12.7 mM hydroxylamine, with the addition of 61.5 mM iron (III) chloride and 3.3 mM 1,10-phenanthroline, keeping the sample under the light of lamps to generate superoxide radical, which reduces the content of hydroxylamine by reacting with it at 575 nm [21 with changes] (SP-2000 spectrophotometer, OKB Spectr, Russia). The ratio of tissue: phosphate buffer (pH=7.5) for analysis is 1 to 10, aliquot is 50 µl. The determination of superoxide radical content was performed using a calibration graph within the range of hydroxylamine concentrations of 5 to 200 nM, and the results were expressed in nmol/g FW.

2.3 qRT-PCR

Total RNA extraction was carried out as described in [23, 24]. by CTAB buffer (pH 4.8–5.0); 100 mM Tris-HCl (pH 8.0), 25 mM EDTA, 2M NaCl, 2% CTAB, 2% PVP, and 2% mercaptethanol was added to the mixture before use. Using a chloroform-isoamyl mixture (24:1), the mixture was separated into aqueous and organic phases. Total RNA was precipitated in aqueous phase with absolute isopropanol. Next, to remove genomic DNA from the mixture, the total RNA with DNAse preparation (Syntol, Moscow, Russia) was incubated at 37°C for an hour. Inactivation of DNAse was carried out by heating the mixture at 70°C for 10 minutes before performing the reverse transcription reaction. RT reaction was performed using «T100 Thermalcycler» (BioRad, Foster City, California, USA) with a set of MMLVRT reagents (Evrogen, Moscow, Russia) using Oligo(dT)15-and Random (dN)10-primer. The reaction mixture for PCR (25 µL totally) contained 5 µL qPCRmix-HS SYBR (Evrogen, Moscow, Russia), 1 µL of forward and with Amplex red 1 to 1 in the presence of peroxidase at 570 nm [22 with changes] (Spectro Star Nano spectrophotometer (BMG Labtech, Germany)). The ratio of tissue: phosphate buffer (pH=7.5) for analysis is 1 to 10, aliquot is 15 µl. The hydrogen peroxide content was determined by analyzing a calibration graph within the range of hydrogen peroxide concentrations of 0.1-10 µM based on the optical density, and it was expressed as nmol/g FW.
reverse primers (0.4 µM) (Synthol, Moscow, Russia), 2 µL of template cDNA, and 16 µL of deionized, nuclease-free water. The final content of the cDNA reaction mixture for all samples was ~100 ng. qRT-PCR was performed under the following conditions: 95°C for 5 min for a further 40 cycles, denaturation (95°C, 15 s), annealing (52.7—61.6°C, 30 s), and elongation (72°C, 50 s). For each pair of primers, a negative control was used—PCR was performed in the absence of a cDNA template. The Efla1 gene was used as a reference gene for calculating the relative expression of genes, which, according to analysis using BestKeeper and NormFinder, was the only gene stably expressed in all tissues studied. The primer sequence used for qRT-PCR: Efla1 forward primer (F) 5’TCCTTGAGGCTTTGACTTG3’, reverse primer (R) 5’ATACCAGGCTTGTGACACC3’; BpPAL1(F) 5’GGGAATCCAGCAATTTCG3’, BpPAL1(R) 5’GGCAGACCTGTTCTTTTC3’; BpPAL2.1(F) 5’GAGTCAAGGCGAGCAGTG3’, BpPAL2.1(R) 5’GGTTCTTATGTGAGGATGC3’. 

2.5 Molecular genetic research

Within the scope of the present study, we examined the expression of PAL family genes responsible for encoding phenylalanine ammonia lyase. Additionally, we investigated UPBEAT1 gene, which encodes a transcription factor belonging to the basic/helix-loop-helix (bHLH) family and is involved in the regulation of ROS distribution [25]. Ef1a gene (Bpev01.c0437.g0013.m0001) was used as a reference gene for calculating relative expression [26].

RQ, the relative quantity of gene transcripts, was determined using the formula $RQ = E^{-ΔCt}$, where $ΔCt$ represents the difference in threshold cycle values for the reference and target genes, and $E$ denotes the PCR effectiveness. The effectiveness of PCR was determined individually for each reaction based on amplification fluorescence data using the LinRegPCR software (version 2021.1, dr. J.M. Ruijter, Amsterdam UMC, Amsterdam, Netherlands).

The search for genes of interest was conducted using the silver birch genome, published on the CoGe portal [27]. For this purpose, the amino acid sequences of the proteins of interest from Arabidopsis thaliana UniProt/SwissProt (https://www.uniprot.org) and Populus trichocarpa (PlantGenIE.org) were obtained. The resulting sequences were then used as a BLAST search against the genome of Betula pendula var. pendula (release 1.2,
https://genomevolution.org/coge) to identify homologous sequences.

The structure of silver birch proteins was predicted using the National Center for Biotechnology Information (NCBI) resource [28]. Phylogenetic analysis was performed using the MEGA 11 program [29]. Multiple alignments of silver birch, Arabidopsis, and poplar proteins were performed using ClustalW. Phylogenetic trees were constructed using the nearest neighbor method (Neighbor-Joining method) [30]. The model used to construct the phylogenetic tree of each gene group is indicated in the figure description (Figure 2).

Determination of the percentage identity/similarity of silver birch and poplar proteins was performed in EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/).

A search of the silver birch genome revealed 9 genes encoding homologous Arabidopsis PAL proteins. All 9 sequences contained the phenylalanine ammonia lyase-specific domain PLN02457. Phylogenetic analysis revealed that B. pendula PAL members cluster into three major clades (Figure 2).

**Figure 2.** Phylogenetic tree obtained based on comparative evolutionary analysis of the amino acid sequences of potential PAL proteins of silver birch (Bpev01), proteins of Arabidopsis thaliana and Populus trichocarpa PAL family using the Neighbor-Joining method. The access code for the TAIR (A. thaliana) and Plantgenie (P. trichocarpa) databases is indicated next to the protein names.

A gene search in the silver birch genome revealed only one gene homologous to Arabidopsis UPBEAT1 (AT2G47270) – Bpev01.c0129.g0072.m0001. The sequence contained a conserved domain - basic helix-loop-helix (bHLH) domain (cd11444) and was used to further study the expression level.

Primers for RT-PCR were chosen for all genes using the program Beacon Designer 8.21 (PREMIER Biosoft) (Table 1). For the genes forming the clade - BpPAL3.1 and BpPAL3.2 (Bpev01.c1165.g0003.m0001, Bpev01.c1165.g0004.m0001) and BpPAL4.1/4.2 genes (Bpev01.c0051.g0089.m0001, Bpev01.c0051.g0086.m0001) it was not possible to construct specific primers for RT-PCR because of the high amino acid identity of the genes, so for further PCR, primers that were universal for each pair of sequences were designed. Then the obtained data on the expression of these genes are considered as the total expression of the BpPAL3.1 and BpPAL3.2 genes (in the results referred to as BpPAL3) and the BpPAL4.1 and BpPAL4.2 genes (denoted as BpPAL4). For the remaining genes, sequence-specific primer pairs were designed (Supplementary Table 1).

### 2.6 Mathematical processing

Sample sizes are denoted as n. Data in the diagrams appear as mean ± SE, where SE is the standard error (superoxide radical and hydrogen
peroxide contents, POD active). Expression level data are presented in boxplot format. The results were statistically processed with PAST (version 4.13). Before starting the statistical analysis, the raw data was initially tested for normality using the Shapiro-Wilk test. Paired comparison were made using Mann-Whitney U-test. Principal component analysis (PCA) was carried out for different tissue groups of data set. Before the calculations, the initial data were standardized.

All the differences discussed were significant at p <0.05.

All assays were performed at the Core Facility of the Karelian Research Centre RAS.

3. RESULTS

3.1 UPBEAT1 and PAL expression

Figure 3 shows the expression level of UPBEAT1 was higher in F plants in all tissue complexes studied. The highest expression was observed in F2.

PAL1 and PAL2.1 were higher in p plants in F2, PAL1, PAL2.2, and PAL4 were higher in F plants in MX. PAL3 was higher in F plants in F1, PAL3 and PAL4 were higher in F plants in F2.

Figure 3. Expression level of the studied genes (UPBEAT1, PAL1, PAL2.1, PAL2.2, PAL3, PAL4) in common silver birches (p), figured wood birches (F) inside certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.

3.2 Superoxide radical and hydrogen peroxide content

The superoxide radical content was predominant in F plants in F1. There were no significant differences between p and F in hydrogen peroxide content, however, the ratio of superoxide radical to hydrogen peroxide tends to be higher on average in F plants (Figure 4).

Figure 4. Superoxide radical content, hydrogen peroxide content, and superoxide radical / hydrogen peroxide ratio in common silver birches (p), figured wood birches (F) inside certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.

3.3 Peroxidase activity

The activity of POD, which indirectly indicates the balance of superoxide radical and hydrogen peroxide, exhibited higher levels in F plants in F1 and F2 tissue complexes (Figure 5).

Figure 5. Peroxidase activity in common silver birches (p), figured wood birches (F) inside
certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.

3.4 Principal Component Analysis

Principal component analysis (PCA) was performed in all the studied data in all the studied tissue complexes separately. Its results confirmed that investigated trees (p, F) were divided into separate groups based on the studied parameters.

In F1 factor 1 (53.6 % of the variance) was correlated with PAL3 (r = 0.50), PAL4 (r = 0.45), POD (r = 0.42), UPBEAT1 (r = 0.42), superoxide radical (r = 0.33). Factor 2 (24.5 % of the variance) was correlated with PAL1 (r = 0.60), PAL2.2 (r = 0.52), PAL2.1 (r = 0.36). In F2 – included differentiating early xylem cells. Factor 1 (63.3 % of the variance) was correlated with PAL2.1 (r = -0.50), UPBEAT1 (r = 0.41), PAL4 (r = 0.40), POD (r = 0.38), PAL3 (r = 0.35). Factor 2 (12.2 % of the variance) was correlated with PAL2.1 (r = -0.55), PAL2.2 (r = 0.50), PAL1 (r = 0.48). In MX – included cells of mature xylem. Factor 1 (47.4 % of the variance) was correlated with PAL2.1 (r = -0.57), UPBEAT1 (r = -0.42), PAL4 (r = 0.38), superoxide radical / hydrogen peroxide ratio (r = 0.35), PAL1 (r = -0.35), UPBEAT1 (r = 0.34). The blue group represents common silver birches (p), and the red group represents the parts of figured wood birches with figure signs (F).

4. DISCUSSION

Recent studies have noted the significant role of ROS in the xylogenesis processes. But on the other hand, much remains unknown about how vascular cambium proliferates with ROS participation [31, 32].

In this investigation PCA showed that the studied plants form separate groups according to the investigated indicators (transcription factor UPBEAT1, genes of the PAL family, POD activity, included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone. Factor 1 (53.6 % of the variance) was correlated with PAL3 (r = 0.50), PAL4 (r = 0.45), POD (r = 0.42), UPBEAT1 (r = 0.42), superoxide radical (r = 0.33). Factor 2 (24.5 % of the variance) was correlated with PAL1 (r = 0.60), PAL2.2 (r = 0.52), PAL2.1 (r = 0.36). In F2 – included differentiating early xylem cells. Factor 1 (63.3 % of the variance) was correlated with PAL2.1 (r = -0.50), UPBEAT1 (r = 0.41), PAL4 (r = 0.40), POD (r = 0.38), PAL3 (r = 0.35). Factor 2 (12.2 % of the variance) was correlated with PAL2.1 (r = -0.55), PAL2.2 (r = 0.50), PAL1 (r = 0.48). In MX – included cells of mature xylem. Factor 1 (47.4 % of the variance) was correlated with PAL2.1 (r = -0.57), UPBEAT1 (r = -0.42), PAL4 (r = 0.38), superoxide radical / hydrogen peroxide ratio (r = 0.35), PAL1 (r = -0.35), UPBEAT1 (r = 0.34). The blue group represents common silver birches (p), and the red group represents the parts of figured wood birches with figure signs (F).

Figure 6. Scheme ordination of 10 B. pendula trees on all the studied data in F1 –
content of hydrogen peroxide and superoxide radical) (Figure 6). The differences that were observed were particularly noticeable in the thin layer of differentiating xylem. It is known that differentiating xylem elements are capable of sustaining the production of ROS [33]. When planning the study, we assumed that the studied transcription factor UPBEAT1 may have an indicator role near the division of cambial derivatives area and found that UPBEAT1 expression was higher in all tissue complexes studied in figured Karelian birch plants. It is then logical that in each tissue fraction studied, the ratio of superoxide radical and hydrogen peroxide, on average, was higher in F plants, confirming that proliferation during the formation of figured wood (high content of parenchyma cells) prevails over differentiation (vessels and fibers formation) in Karelian birch plants, as we noted earlier in our biochemical, anatomical and molecular genetic studies [4, 17, 18, 20, 26, 34-39]. Earlier in recent literature it was also noted that UPBEAT1 controls the ratio of differentiation and proliferation processes, including through its influence on the POD expression [40, 41].

The peroxidase activity in Karelian birch plants is observed to be higher (Figure 6). It is assumed that this enzyme, along with other AOS and secondary metabolism enzymes [42], actively participates in altering the balance between proliferation and differentiation in plants with figured wood. Nonetheless, the consistent concentration of hydrogen peroxide (Figure 4) along the radial series suggests its potential involvement in differentiation processes in p plants [43, 44]. Furthermore, in F plants, it is generated as a byproduct of the superoxide dismutase reaction, as indicated by the elevated CAT and POD activity [42]. Hence, in the processes being examined, it is crucial to focus not on the absolute ROS content being studied, but rather on the ratio between superoxide radical and hydrogen peroxide, which is regulated by the transcription factor UPBEAT1 (Figure 3).

The key enzyme of secondary metabolism is phenylalanine ammonia lyase [45]. It is known that the PAL gene family in poplar (Populus trichocarpa) consists of 5 genes (PtrPAL1–5), which are divided by phylogenetic analysis into 2 groups. Members of the first group (PtrPAL2, 4 and 5) are mainly expressed in xylem and roots, while members of the second group (PtrPAL1 and 3) are more widely represented [46]. In poplar, this clear difference in PAL gene expression, coupled with the colocalization of 4-coumarate-CoA ligase 2 (Ptr4CL2) and condensed tannins with PtrPAL1, suggests that PtrPAL1 and 3 are primarily responsible for the formation of condensed tannins, flavonoids, and other phenolic compounds [46, 47].

Interestingly, the relative expression levels of BpPAL3 and BpPAL4, which are identical to PtrPAL1 and PtrPAL3, respectively (Figure 3), exhibited higher levels in F plants, particularly in the thin layer of differentiating xylem (F2). This further confirms the transition towards secondary metabolism during the predominance of parenchymatization processes in Karelian birch
plants. In turn, PAL and POD are involved in the synthesis of lignin [48], the content of which, as was previously shown above, in the xylem of Karelian birch [17].

Thus, in this work, for the first time in the radial raw cambial zone - differentiating xylem - mature xylem, ROS content (superoxide radical and hydrogen peroxide) was determined, along with data on the peroxidase activity, which we also received earlier [17-18], but these data were considered for the first time in the radial raw, showing that F1 and F2 tissues contribute to its indicator role in Karelian birch during the period of active growth. It was shown for the first time that during different xylogenesis pathways: either more intense differentiation (in the straight-layered wood formation), or more intense proliferation of parenchyma cells that form the anatomical basis of figured wood 1) there is a shift in the balance of superoxide radical and hydrogen peroxide, which are under the control of the transcription factor UPBEAT1, and 2) control of the shift of differentiation towards proliferation under the control of PAL genes. Furthermore, previous research has demonstrated that the transcription factor UPBEAT1, as described in the literature [1, 2], has a direct impact on POD activity. This factor supports the substrate and signaling functions of hydrogen peroxide and superoxide radicals, influencing the differentiation of cambial derivatives in a unique model organism. Earlier research [17] revealed that, in Karelian birch, the formation of structural anomalies triggers an enhancement in the antioxidant system, resulting in an increased substrate load on ROS. An increase in superoxide radical levels, controlled by UPBEAT1, was identified as the cause of intensified proliferation in Karelian birch. The processes under study were examined for the first time in a complex on a woody plant with lateral meristem deviations.

**CONCLUSION**

An increase in the content of superoxide radical has been shown, along with an increase in the transcription's factor UPBEAT1 expression, as well as the expression of PAL genes, which handle the formation of phenolic compounds in Karelian birch compared to silver birch. For the first time, the study was carried out not entirely in xylem and phloem, but in a radial tissue row: F1-F2-MX. We have shown that the processes occurring in a thin layer of differentiating xylem (F2) have a diagnostic role for the visually observed changes in the xylogenesis during the figured wood formation in Karelian birch. The metabolic picture confirms the shift of xylogenesis in Karelian birch towards proliferation processes, accompanied by ROS and phenolic compounds flow, involving antioxidant system and secondary metabolism enzymes in intensive work. In general, we would call this a special type of UPBEAT1-ROS-POD-PAL system functioning which characterizes some cause-and-effect metabolic relationships under different xylogenesis’ scenarios in Karelian birch.

**LIST OF ABBREVIATIONS**

PAL = Phenylalanine ammonia lyase
SOD = Superoxide dismutase
CAT = Catalase
POD = Peroxidase
ROS = Reactive oxygen species
AOS = Antioxidant system

AUTHORS’ CONTRIBUTION
Kseniya M. Nikerova: Conceptualization, Supervision, Methodology, Writing - original draft. Natalia A. Galibina: Conceptualization, Writing - review & editing. Irina N. Sofronova: Methodology, Biochemical analysis. Anna V. Klimova: Biochemical analysis, Writing - review & editing. Yulia L. Moshchenskaya, Maksim A. Korzhenevskij, Tatiana V. Tarelkina: Molecular genetic analysis, Writing - review & editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

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No animals/humans were used for studies that are the basis of this research.

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Not applicable.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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Figure 1. Debarked surface common silver birches (*B. pendula* var. *pendula* straight-grained trees) (p), figured birches (*B. pendula* var. *carelica* trees) (F) and tissue complexes, included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone (F1); the layers of tissue, including differentiating early xylem cells (F2), and cells of the mature xylem (MX).

Bar – 500 µM.
Figure 2. Phylogenetic tree obtained based on comparative evolutionary analysis of the amino acid sequences of potential PAL proteins of silver birch (Bpev01), proteins of Arabidopsis thaliana and Populus trichocarpa PAL family using the Neighbor-Joining method. The access code for the TAIR (A. thaliana) and Plantgenie (P. trichocarpa) databases is indicated next to the protein names.
Figure 3. Expression level of the studied genes (UPBEAT1, PAL1, PAL2.1, PAL2.2, PAL3, PAL4) in common silver birches (p), figured wood birches (F) inside certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.
Figure 4. Superoxide radical content, hydrogen peroxide content, and superoxide radical / hydrogen peroxide ratio in common silver birches (p), figured wood birches (F) inside certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.
Figure 5. Peroxidase activity in common silver birches (p), figured wood birches (F) inside certain tissue complex: F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone, F2 – included differentiating early xylem cells and MX – included cells of mature xylem. Latin letters indicate significant differences (Mann-Whitney U-test) between p and F samples inside tissue complexes.
Figure 6. Scheme ordination of 10 *B. pendula* trees on all the studied data in F1 – included cells of non-conductive phloem, fully formed early phloem, differentiating late phloem, and cambial zone. Factor 1 (53.6 % of the variance) was correlated with PAL3 (r = 0.50), PAL4 (r = 0.45), POD (r = 0.42), UPBEAT1 (r = 0.42), superoxide radical (r = 0.33). Factor 2 (24.5 % of the variance) was correlated with PAL1 (r = 0.60), PAL2.2 (r = 0.52), PAL2.1 (r = 0.36). In F2 – included differentiating early xylem cells. Factor 1 (63.3 % of the variance) was correlated with PAL2.1 (r = -0.50), UPBEAT1 (r = 0.41), PAL4 (r = 0.40), POD (r = 0.38), PAL3 (r = 0.35). Factor 2 (12.2 % of the variance) was correlated with PAL2.1 (r = -0.55), PAL2.2 (r = 0.50), PAL1 (r = 0.48). In MX – included cells of mature xylem. Factor 1 (47.4 % of the variance) was correlated with PAL2.1 (r = -0.57), UPBEAT1 (r = -0.42), PAL4 (r = 0.38), superoxide radical / hydrogen peroxide ratio (r = 0.35), PAL1 (r = -0.35), UPBEAT1 (r = 0.34). The blue group represents common silver birches (p), and the red group represents the parts of figured wood birches with figure signs (F).