



# Compounds composition of pollen tubes of Scots pine (*Pinus sylvestris* L.)

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## Abstract

The aim of this research was to study the composition of pollen tubes of Scots pine (*Pinus sylvestris* L.). Pollen cultivation on deionized distilled water excluded the potential influence of the cultivation medium on the pollen tube growth and development. The fluorescent study indicated a gradual distribution of chemical compounds along the length of the tube. It was shown that the protoplast apical zone and the parietal layer near the tube's tip are most likely actively involved in the ion transport regulation in the growing pollen tube. The callose synthesis in the tip of matured pine tube completed the first stage of its active growth. Significant differences and pH gradients at the nucleus region and the parietal layer of the tube wall indicate that H<sup>+</sup> gradient is the direct driving force of vesicle transport and can regulate the growth of pollen tubes. The distribution of amino acids, RNA, proteins and lipids was uniform throughout the length of the pine pollen tube. The content of amino acids, RNA, DNA and proteins slightly increased near the cell nucleus and drastically increased in the apical zone. At the very tip of the tube, a slight increase in the concentration of polysaccharides and a significant decrease in the content of amino acids, RNA, DNA, proteins and lipids were detected.

**Keywords** *Pinus sylvestris* · Compounds composition · Pollen tubes

## Abbreviations

DAPI	4',6-Diamidino-2-phenylindole
DTIR	Disturbed total internal reflection
Fluorescein-DHPE	N-(Fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt
FTIR	Fourier-transform infrared spectroscopy
IR spectra	Infrared spectra
MQAE	N-(Ethoxycarbonylmethyl)-6-methoxyquinolinium bromide

## Introduction

Pollen tubes play an important role in the sexual reproduction of seed plants, delivering male gametes to eggs. By numerous characteristics, the pollen tube is not a typical plant cell. The polar growth of the pollen tube is caused by the transport of vesicles into the apical zone, where they merge with a narrow section of the plasma membrane (Onelli and Moscatelli, 2013). The mechanism behind the polar growth and vesicle transport remains poorly understood. The main challenge for researchers here is that tube growth in vitro often does not correlate to its growth in vivo. In addition, the composition of the nutrient medium used for pollen germination in vitro affects the metabolism of pollen tubes (da Costa et al. 2013). Even in optimized media, pollen tubes in vitro in most cases reach only 30–40% of their length in vivo and often display structural anomalies. However, in some experiments, in vitro studies can well correlate with in vivo tube growth (Taylor and Hepler 1997).

Since the first discovery of pollen tubes by Amici, a significant amount of experimental data has been accumulated on various aspects of the growth and development of pollen tubes, mostly related to angiosperm plants. After sequencing the genome of *Arabidopsis thaliana* (L.) Heynh. in 1990s,

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many researches have adopted it as a model in molecular genetics and physiological studies (Higashiyama and Inatsugi, 2006). The number of works on the biology of gymnosperm pollen tubes is not large, although significant attention has recently been paid to this group of plants (Breygina et al. 2021).

Structurally and functionally, pollen tubes represent complex multicomponent relatively autonomous biological systems.

In most angiosperm species, the wall of the pollen tube consists of two layers; the inner layer contains mainly callose, and the outer layer consists of pectin, cellulose and hemicelluloses. The growing tip of the pollen tube, where new components of the cell wall are constantly synthesized, contains pectin providing its rigidity and, at the same time, plasticity, and contributing to the directed growth of the tube (Krichevsky et al. 2007). Cellulose, despite its low content in the pollen tube wall, plays an important role in its growth, determining the direction of growth and limiting the diameter of the tube (Domozych et al. 2013).

Ion fluxes and signaling play an important role in controlling the growth and morphogenesis of pollen tubes (Liu et al. 2010; Tavares et al. 2011; Malhó et al. 2015; Michard et al. 2017). The most important ions are  $\text{Ca}^{2+}$ ,  $\text{H}^+$ ,  $\text{K}^+$  and potentially  $\text{Cl}^-$  (Hepler et al. 2006). The importance of  $\text{K}^+$  and, especially  $\text{Ca}^{2+}$  ions in cellular polarization and differentiation is indicated by the fact that the presence of these ions in the germination medium is necessary to induce electric current in the pollen tubes of certain angiosperms (Pierson et al. 1993; Hepler et al. 2012). In addition to  $\text{K}^+$  and  $\text{Ca}^{2+}$ , the growth of pollen tubes is regulated by pH (Winship et al. 2017). Acidic conditions are necessary for pollen germination in most plant species. Studies on the  $\text{H}^+$  intracellular distribution have shown that pollen tubes have a unique intracellular pH gradient (Feijó et al. 1999). Growing pollen tubes possess a constitutive alkaline band in the clear zone and a growth-dependent acidic tip. However, due to the high mobility of  $\text{H}^+$  ions, these gradients are difficult to visualize (Fricker et al. 1997).

While the significance of  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{H}^+$  for the growth and differentiation of pollen tubes is well established, the role of  $\text{Cl}^-$  is less clear. M.A. Messerli et al. (2004) believe that fluctuations of  $\text{Cl}^-$  concentration are insufficient for the growth of pollen tubes, and  $\text{Cl}^-$  itself is not required for their growth. However,  $\text{Cl}^-$  fluxes form the loop in the apical part of the pollen tube similar to  $\text{H}^+$  ions and thus can contribute to tube polar growth (Zonia et al., 2002). The initiation of pollen grain germination is also associated with the activation of  $\text{Cl}^-$  output streams (Matveeva et al. 2003; Breygina et al. 2012).

Despite the fact that plant cells with polar growth share many similar features, the mechanisms of regulation of the growth and development of pollen tubes in different

taxonomic groups and even in individual species within the same taxon can vary greatly.

The morphogenesis of the microgametophyte in Pinaceae has been studied in detail (Kozubov et al. 1982; Fernando et al. 2005). Many researchers have addressed various aspects of the growth and development of pollen tubes in genus *Pinus*. However, the mechanisms of regulation of these processes still remain insufficiently studied.

The aim of the work was to study the distribution of organic compounds and major ions in pollen tubes of Scots pine. Pollen cultivation on  $\text{diH}_2\text{O}$  made it possible to exclude the potential influence of the medium on the growth and development of pine pollen tubes.

## Materials and methods

### Light and scanning electron microscopy

Pollen was germinated on  $\text{diH}_2\text{O}$  using the hanging drop method (Pausheva 1988) and in Petri dishes on 1% agar with the addition of 5% sucrose for 96 hours in a thermostat at + 26.5 °C. Deionized water with a resistivity of 18.2  $\text{M}\Omega/\text{cm}$  was obtained using a Simplicity UV system (Millipore, Molsheim, France). Intravital staining with Calcofluor, Fluo-4 AM ester, SAE and Fluorescein-DHPE was performed according to Biotium (USA) protocols. DAPI staining was carried out according to the method described in Yu. Cao et al. (2012). Pollen tubes were stained also with aqueous solutions of I2-KI and methyl green - pyronine G using well known methods (Jensen 1962). The exposure time during staining was selected empirically. The stained tubes were visualized using an AxioScope A1 direct microscope and an Altami Lum ILED fluorescence microscope.

The gradient of chemical elements along the pollen tube length was determined using the EDX-microanalysis on a scanning electron microscope ZEISS SEM-Sigma VP. Freeze-dried tubes were scanned without additional sputter coating with an accelerating voltage of 20 kV, an aperture of 30  $\mu\text{m}$ , and an 80  $\text{mm}^2$  Oxford detector. The concentrations of the elements were determined relative to oxygen, the oxygen concentration was taken as 100%.

### FTIR spectroscopy

A sample of pine pollen tubes was prepared for analysis as follows. Pollen was germinated in hanging drop in  $\text{diH}_2\text{O}$  for 96 h at + 26.5 °C. Then the aqueous suspension of germinated pollen was frozen at a temperature of – 80 °C and freeze-dried. Samples of mature pollen grains were purified by sieving through a fine sieve and used for analysis without additional processing.

FTIR spectra of samples were recorded on the IR Prestige 21 Fourier spectrometer (Shimadzu, Japan) combined with MIRacle ZnSe prism (Pike, USA) for Attenuated Total Reflection. The spectra were recorded within the range of 4,000–600  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  with 128 scans for each spectrum and Sqr Triangle apodization function. The background spectrum was obtained against the air. The obtained values of the transmission coefficients were converted into units of optical density by processing the obtained spectra with the ATP-correction function using the IR Solution software.

The relative optical densities (K) were used to estimate the chemical composition. The K values were calculated using an internal standard relative to the baseline by wave numbers of 4,000; 3,800; 2,400; 1,800; 800, 600  $\text{cm}^{-1}$ .

$$K_i = \frac{A_i}{A_{st}}$$

where  $A_i$ ,  $A_{st}$  are the intensities of the characteristic absorption band of the component and the internal standard (2,924  $\text{cm}^{-1}$  corresponding to C-H stretching in all components).

IR studies of pine pollen tubes along their length were carried out on the Vertex 70v infrared Fourier spectrometer (Bruker, Germany) using the HYPERION 3000 infrared microscope (Bruker, Germany). A point-based MCT detector was used, which allows taking infrared spectra at a point of 1  $\mu\text{m}$  in size. The spectra were recorded with aluminum foil background in reflection mode with 36x-IR lens. Scanning range was from 4,000 to 600  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  and 1,024 scans for each positioning point. Twenty equidistant points were scanned in each tube. The first point was as close as possible to the distal aperture of the pollen grain without entering the grain itself, the last point was as close as possible to the tip of the tube without leaving it. The absorption band at 2,920  $\text{cm}^{-1}$  was chosen as an internal standard.

### Pyrolysis gas chromatography—mass spectrometry (Py-GC-MS)

Py-GC-MS analysis was carried out on a QP-2010 Plus gas chromatography—mass spectrometry system (Shimadzu, Kyoto, Japan) equipped with an EGA/PY-3030D pyrolyzer (Frontier Lab, Koriyama, Japan) and a liquid nitrogen cryotrap. The samples were prepared similarly to the FT-IR spectroscopy. The dry samples (1.8–2.7 mg) were placed in stainless steel micro crucibles and subjected to the thermal decomposition in helium atmosphere by heating from 50 to 600  $^{\circ}\text{C}$  at a rate of 50  $^{\circ}\text{C}\cdot\text{min}^{-1}$ . The chromatographic separation of the pyrolysis products was carried out on an HP-5ms capillary column (Agilent, Santa Clara, USA) 30 m  $\times$  0.25 mm, with a film thickness of 0.25  $\mu\text{m}$ . High-purity helium with a flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$  was used as a carrier gas. The column temperature was programmed as follows:

40  $^{\circ}\text{C}$ , held for 2 min; followed by a 3  $^{\circ}\text{C}\cdot\text{min}^{-1}$  linear ramp to 320  $^{\circ}\text{C}$ , held for 5 min. Mass spectrometry detection was performed with electron ionization (70 eV) in a scanning mode in a range of 35–600 Da and at a scanning speed of 2  $\text{kDa}\cdot\text{s}^{-1}$ . The detected compounds were identified using the NIST/Wiley 2011 library.

## Results and discussion

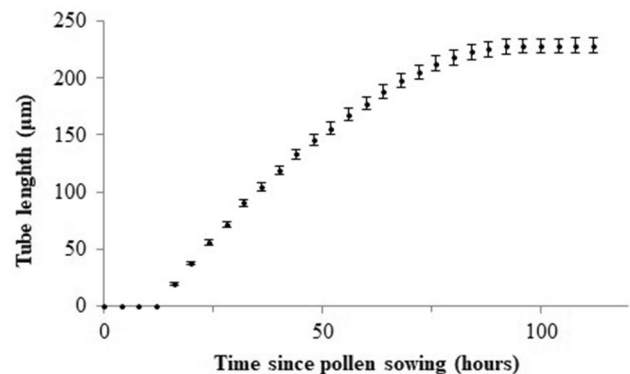
The hydration of pine pollen grains *in vitro* took 12–14 hours, followed by the intine protrusion through the distal furrow and the active growth of the pollen tube. The pollen tube growth rate remained constant for the first 60 hours, then it gradually slowed down, and after 96 hours from sowing, there was no visual growth (Fig. 1).

The oscillatory growth behavior have been found in pollen tubes of many flowering plants (Messerli, Robinson, 2003); it is probably also typical for conifers but to a lesser extend.

The average length of pollen tubes of Scots pine *in vitro* rarely exceeds 250–300  $\mu\text{m}$ . However, they displayed changes similar to those in male gametophyte *in situ* in the year of pollination. The tube cell nucleus with associated protoplast moved into the pollen tube, while the generative nucleus remained inside the grain body (Fig. 2a, b).

Water-soluble sugars serve as a source of carbohydrates for building the body of the pollen tube, mainly its walls. Sucrose is commonly used to germinate pollen on artificial media. According to our data, pollen tubes of Scots pine grow better on media containing galactose (Table 1).

During the germination in sugar media excessive sugars can cause starch accumulation in amyloplasts, which can occupy a significant volume of the tube cell or completely fill it (Fig. 2c, d), making it difficult to visualize the structural changes inside the growing tubes. Freshly collected pine pollen, unlike most coniferous species, germinates



**Fig. 1** Growth dynamics of Scots pine pollen tubes on agar medium with 5% sucrose (the interval between measurements was 4 h)

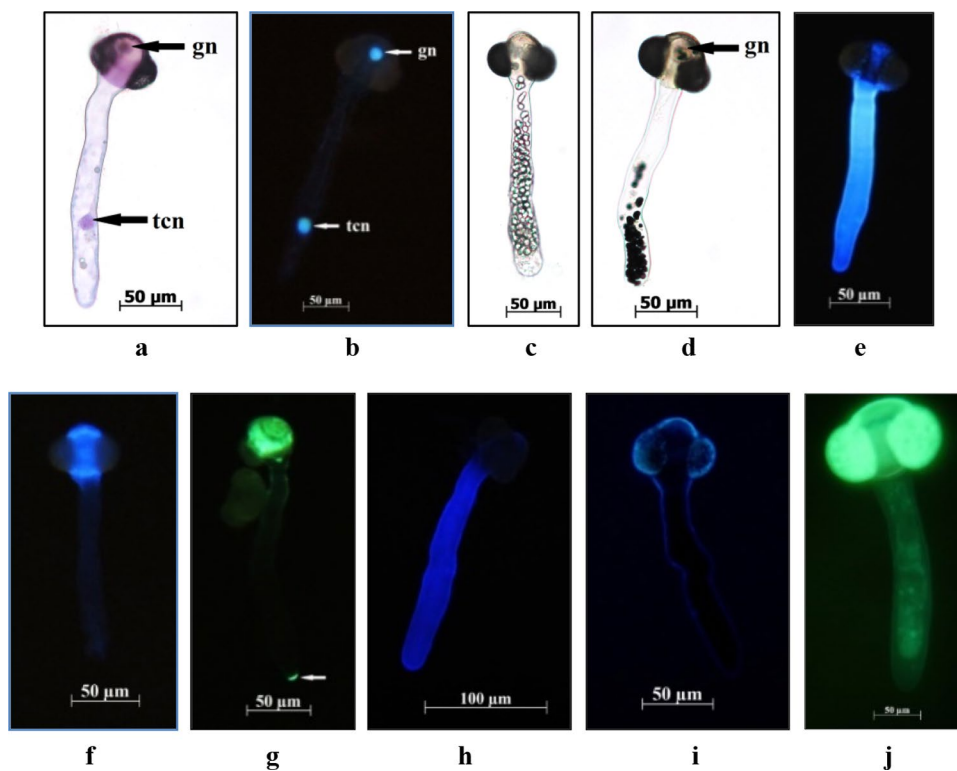
well in distilled water, forming pollen tubes as long as tubes germinated on sugar media (Fig. 2a). Staining with I<sub>2</sub>-KI solution did not reveal any starch grain in pollen tubes germinated in distilled water.

The staining of pine pollen tubes with Calcofluor, a blue fluorescent dye binding to cellulose, resulted in the fluorescence intensity gradually decreased towards the tube tip (Fig. 2e). This fluorescence pattern indicates a gradual differentiation of the tube wall in the direction from its tip. After incubation of the tubes with cellulase for 24 hours, the Calcofluor fluorescence was not observed any more in the tubes (Fig. 2f). Tube incubation with cellulase and

pectinase for 48 hours completely destroyed the shells of pollen tubes.

Callose is the main polysaccharide of the pollen tube wall in angiosperms, it is localized in the inner layer and absent in the tube tip (Mollet et al. 2013). On the contrary, in many coniferous species, including *Pinus* genus, callose was not detected in the pollen tube wall by aniline blue staining (Yatomi et al. 2002). However, J. Derksen et al. (1999) found strong fluorescence in the tip of pollen tubes of *P. sylvestris* when they were stained with aniline blue. Our results confirmed these observations. We observed a bright fluorescence only at the very tip of the pine tube stained with aniline blue (Fig. 2g).

**Fig. 2** Pollen tubes of Scots pine after in vitro cultivation for 96 hours at +26.5 °C. (a, b, e–j) germinated in diH<sub>2</sub>O by the “hanging drop” method, (c, d) germinated on 1% agar with 5% sucrose; a stained with methyl green—pyronine G, b DAPI staining, c without staining, d I<sub>2</sub>-KI staining, e, f Calcofluor (f after additional incubation with cellulase for 24 h), g aniline blue staining (the arrow shows the callose fluorescence at the tube tip), h Fluo-4 AM ester, i MQAE, j fluorescein-DHPE gn—generative nucleus, tcn—tube cell nucleus



**Table 1** The effect of individual sugars on pine pollen germination and pollen tubes growth in vitro (the concentration of each of the sugars in the agar medium was 5%). With a given accuracy  $P = 0.95$ , the standard errors of the averages did not exceed the permissible values

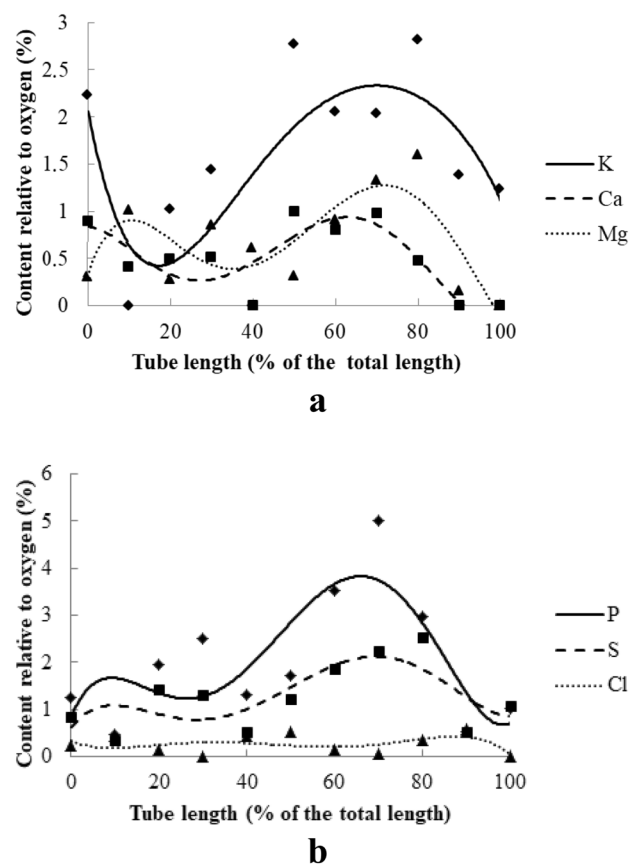
Name of sugars and control test	Pollen viability, %	The average length of the pollen tube, $\mu\text{m}$	The maximum length of pollen tube, $\mu\text{m}$
sucrose	$84.5 \pm 8.5$	$173.8 \pm 6.6$	$237.5 \pm 8.3$
glucose	$79.5 \pm 7.8$	$167.5 \pm 12.0$	$226.3 \pm 13.4$
raffinose	$37.5 \pm 9.7$	$105.0 \pm 8.7$	$152.5 \pm 11.1$
fructose	$52.5 \pm 9.5$	$61.3 \pm 14.2$	$92.5 \pm 17.1$
maltose	$3.5 \pm 2.3$	$33.8 \pm 13.4$	$62.5 \pm 21.7$
mannose	0.0	-	-
galactose	$96.3 \pm 3.1$	$205.0 \pm 15.5$	$255.0 \pm 10.4$
cellobiose	$47.5 \pm 6.0$	$73.8 \pm 17.0$	$113.8 \pm 18.9$
diH <sub>2</sub> O (Control test)	$54.4 \pm 7.6$	$112.3 \pm 6.5$	$197.7 \pm 6.1$

Freshly collected pine pollen germinates perfectly in distilled H<sub>2</sub>O. In this case, all chemical compounds enter the growing pollen tube from the hydrated pollen grain. According to our data, mature pine pollen contains, on average, 1444.8 µg/g of magnesium, 543.8 µg/g of calcium, 111.6 µg/g of manganese, 34.7 µg/g of iron, 22.4 µg/g of zinc, 2.8 µg/g of copper, 1.2 µg/g of selenium. The content of other elements (except hydrogen, oxygen and carbon) does not exceed 1 µg/g. This amount seems to be sufficient to provide the endogenous fluctuation of ions necessary for the normal growth of tubes, at least at the first stage of their development.

Figure 3 shows characteristic distribution of chemical elements along the Scots pine pollen tubes from the distal furrow of germination to the tube tip.

The distribution of potassium, calcium and magnesium was largely similar; their concentration first decreased when moving from the distal furrow of the pollen grain, and then increased, reaching maxima in the nucleus region, and decreased again closer to the tube tip. These changes were more pronounced for potassium, which has a lower valence and higher concentrations. The gradient of calcium is less visible, which corresponded to the Fluoro-4 AM staining results (Fig. 2h). Fluoro-4 AM is a membrane permeable ethereal form of Fluoro-4 and it provides a high fluorescence signal. The chlorine concentration changes along the tube length were negligible. Staining with MQAE dye, which has a very high sensitivity to Cl<sup>-</sup>, resulted in approximately equal fluorescence intensity along the entire tube, noticeably decreasing only in the very tip of the tube (Fig. 2i). The highest concentrations of phosphorus and sulfur were observed in the nucleus zone of the protoplast. The fluorescence intensity of fluorescein-DHPE, a surface pH indicator, decreases with increasing acidity of the medium (Watanabe et al. 2014). According to our observations, this dye is able to penetrate through the tube wall and the outer membranes of cells and cellular organelles inside the tube. The fluorescence intensity of fluorescein-DHPE in pine tubes increased towards the growing tube tip. The fluorescence intensity of the parietal layer of the wall tube did not change much, except for the tube tip itself, where it was the lowest (Fig. 2j).

The EDX-microanalysis and dye staining results gave a general distribution of major ions in pollen tubes. However, significant pH differences throughout the length of the pollen tube indicate that the apical zone and the parietal layer of the wall tube near the tube tip are most likely actively involved in the regulation of ion transport in the growing pine pollen tube. Significant differences in the pH of the protoplast and the layer of the wall tube indicated that the fluctuation of H<sup>+</sup> can play an important role in vesicular transport. The generation of an electrochemical proton gradient is the direct driving force of vesicle transport and can



**Fig. 3** Distribution of chemical elements in pine pollen tubes from the distal furrow (0% of the total length) to the tube tip (100% of the total length) (**a** cations, **b** anions). Freshly collected pine pollen was germinated on deionized distilled H<sub>2</sub>O

regulate the growth of pollen tubes in a greater extent than the Ca<sup>2+</sup> ion gradient (Schuldiner et al. 1978; Winship et al. 2017).

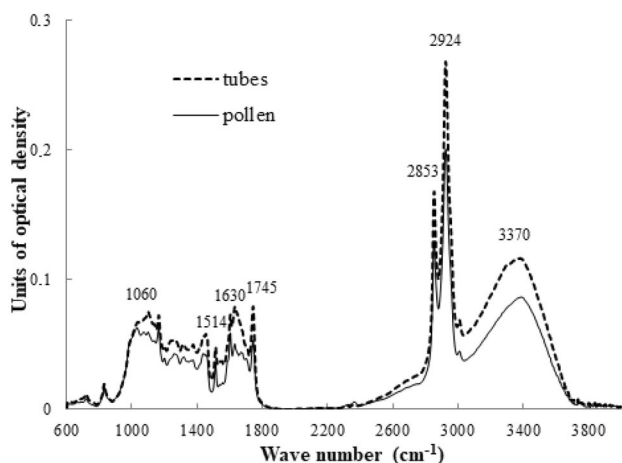
Recently, FTIR spectroscopy methods have been widely used to analyze the composition of pollen and pollen tubes, which make it possible to interpret certain peaks of infrared spectra (Lahlali et al. 2014; Zimmermann 2018).

Coniferous pollen contain a significant amount of non-polar fats, waxes, as well as carotenoids with hydroxyl groups, while pollen tubes contain a relatively high amounts of carbohydrates (mostly starch), proteins, and amino acids (Surso et al. 2020). Thus, the differences in the component composition of pollen grains and pollen tubes determine their specific physiological properties.

The DT-IR spectra of mature not hydrated pollen grains and germinated pollen tubes of pine had a similar shape and the same set of absorption bands, which indicated the similarity of their functional nature (Fig. 4).

Figure 4 represents an example of the typical infrared spectra of pollen and pollen tubes. Based on these spectra,





**Fig. 4** DT-IR spectra of pine pollen and pollen tubes. The absorption band at  $3420\text{ cm}^{-1}$  corresponds to stretching vibrations of hydroxyl groups involved in hydrogen bonds;  $2920$  and  $2853\text{ cm}^{-1}$  is assigned to valence vibrations of C-H bonds,  $1745\text{ cm}^{-1}$  is assigned to valence vibrations of C=O bonds;  $1600$ ,  $1510$ ,  $1425\text{ cm}^{-1}$  are assigned to skeletal vibrations of aromatic rings;  $1650$ – $1630\text{ cm}^{-1}$  are attributed to valence vibrations of hydroxyl groups of bound water. Among the bands at  $1030$ ,  $1060$ ,  $1100\text{ cm}^{-1}$  corresponding to valence vibrations of C-O bonds in simple ethers, the band at  $1060\text{ cm}^{-1}$  is attributed to the polysaccharide component (Vartanian et al. 2015)

conclusions were drawn about the qualitative similarity of the component composition of pollen and pine pollen tubes. Calculation of relative optical densities made it possible to identify quantitative differences in the component composition of pollen and pollen tubes of pine.

The values of the relative optical density coefficients are given in Table 2.

The results of FT-IR spectroscopy along the length of pine pollen tubes in the direction from the distal furrow of germination (0%) to the tube tip (100%) are shown in Fig. 5.

Comparing to pollen grains pollen tubes spectrum had a slightly higher content of bound water hydroxyl groups, which indicates their greater hydrophilicity (EMU notes in mineralogy 2004). In addition, they had a higher content of aromatic and carbonyl structures (proteins, RNA, DNA, lipids) and a relatively lower content of carbohydrates. These

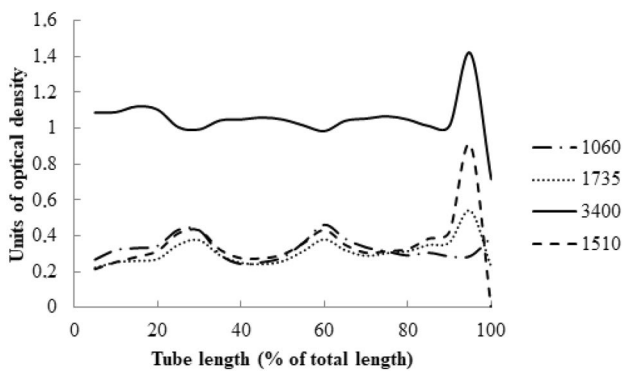
results were also confirmed by the pyrolysis data. The content of identified aliphatic hydrocarbons in pollen and pollen tubes of Scots pine was 16.90 and 6.19%, DNA and proteins – 0.90 and 2.00, aromatic hydrocarbons – 0.20 and 3.35, lignin – 1.02 and 0.42, waxes – 5.26 and 2.16, tannins – 0.51 and 0.27, fats (lipids and phospholipids) – 0.45 and 2.09, steroids – 0.56 and 0.37%, respectively. It should be noted that the distribution of amino acids, RNA, proteins, lipids and polysaccharides was uniform along the pollen tube. A slight increase in the content of amino acids, RNA, DNA and proteins was observed in the region of the tube cell nucleus and a significant increase was observed in the apical zone of the tube cell protoplast. At the very tip of the tube there was a slight increase in the concentration of polysaccharides and a sharp decrease in the content of amino acids, RNA, DNA, proteins and lipids. The obtained results indicate significant differences in the composition of organic compounds in the apical zone of the tube cell protoplast and the clear (optically transparent) zone of the tube tip. Polysaccharides are one of the main components of the tube wall. This, apparently, explains the fact that they are distributed more uniformly along the tube length compared to other components of the tube. These differences were especially pronounced closer to the tube tip.

## Conclusion

Germination of Scots pine pollen in deionized distilled water may be important for studying the physiology of pollen tube growth in vitro. In this study we used various methods to study the component composition of pine pollen tubes. Staining with fluorescent dyes allows visualizing individual components throughout the pollen tube length. Cellulose staining with Calcofluor revealed gradually decrease of its content in the tube wall towards the tube tip. Callose staining with aniline blue resulted in bright fluorescence observed only at the very tip of the tube. Staining with MQAE dye, which has a very high sensitivity to  $\text{Cl}^-$ , gave the approximately equal fluorescence intensity along the entire length

**Table 2** The relative optical densities of pine pollen grains and pollen tubes (obtained from DT-IR spectra)

Sample	Optical densities at certain absorption bands				
	$1060\text{ cm}^{-1}$ C–O–C groups (polysaccharides)	$1514\text{ cm}^{-1}$ aromatic rings (amino acids: tyrosine, phenylalanine, tryptophan and corresponding proteins, phenolic compounds)	$1630\text{ cm}^{-1}$ bound water	$1745\text{ cm}^{-1}$ C=O– groups (proteins, RNA, DNA, lipids)	$3370\text{ cm}^{-1}$ OH– groups carbohy- drates, proteins, RNA, DNA, aromatic and aliphatic alcohols)
Tubes	0.24	0.17	0.29	0.29	0.43
Pollen	0.28	0.16	0.26	0.24	0.42



**Fig. 5** Distribution of groups of organic compounds by the length gradient in the direction from the distal furrow of germination (0%) to the tube tip (100%) of the pine pollen tube according to the results of FT-IR spectroscopy. Absorption bands: 1060  $\text{cm}^{-1}$  (C-O-C bonds in polysaccharides), 1510  $\text{cm}^{-1}$  (amino acids (tyrosine, phenylalanine, tryptophan) and corresponding proteins and possibly aromatic hydrocarbons (phenol)), 1735  $\text{cm}^{-1}$  (C=O bonds in carbonyl and carboxyl groups (proteins, RNA, DNA, lipids)), 3400  $\text{cm}^{-1}$  (O-H groups in carbohydrates, proteins, RNA, DNA)

of the tube, noticeably decreasing only closer to the very tip of the tube. Staining with fluorescein-DHPE, a surface pH indicator, lead to increased fluorescence towards the growing tube tip. The fluorescence intensity of the parietal layer of the tube wall did not change much, except for the tube tip itself, where it is the lowest.

EDX-microanalysis gave an approximate distribution of chemical elements along the length of pollen tubes. The distribution of potassium, calcium and magnesium was similar following the same pattern: a slight decrease from the distal furrow of the pollen grain, followed by maximal concentration in the nucleus zone, followed by a significant decrease in the tube tip. The fluctuation of chlorine along the pollen tube was negligible. The highest concentrations of phosphorus and sulfur were observed in the region of the nucleus.

The composition of organic compounds in pollen and pollen tubes of pine was studied by pyrolytic destruction. The content of identified aliphatic hydrocarbons in pollen and pollen tubes of Scots pine was, respectively, 16.90 and 6.19%, DNA and proteins—0.90 and 2.00, aromatic hydrocarbons—0.20 and 3.35, lignin—1.02 and 0.42, waxes—5.26 and 2.16, tannins—0.51 and 0.27, fats (lipids and phospholipids)—0.45 and 2.09, steroids—0.56 and 0.37%.

The DT-IR spectra of Scots pine pollen grains and tubes demonstrated the same set of absorption bands indicating similarities in their chemical composition. IR analysis along the pollen tube length revealed significant differences in chemical compound distribution. The distribution of amino acids, RNA, proteins, lipids and polysaccharides was almost uniform along the tube length with a slight increase in the nucleus region and a significant increase in the protoplast apical zone. At the very tip of the tube there was a slight

increase in the concentration of polysaccharides and a sharp decrease in the content of amino acids, RNA, DNA, proteins and lipids. The obtained results indicate significant differences in the component composition of organic compounds of the apical zone of the tube cell protoplast and the clear (optically transparent) zone of the tube tip.

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**Data availability** Data sharing does not apply to this article as no datasets were generated or analyzed in the study.

## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

**Human and animal rights** This article does not contain any studies involving humans and animals as research objects.

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