

Research Article

FT-IR Characterization of Pollen Biochemistry, Viability, and Germination Capacity in *Saintpaulia* H. Wendl. Genotypes

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FT-IR characterization of pollen biochemistry was analyzed to detect possible connection with the viability (by staining with potassium iodide, 25%) and the germination capacity (on solid nutrient medium), in 15 *Saintpaulia* genotypes. Vibrational spectroscopy indicates that the pollen of *S. ionantha* genotype “Red Velvet” is rich in proteins, lipids, triglycerides, and esters and has a viability of 88.4% and a low germination capacity (27.16%). For *S. ionantha* “Jolly Red” and “Lucky Ladybug” genotypes, pollen showed high viability (88.81–91.49%) and low germination capacity (23.02–9.17%), even though the pollen is rich in carbohydrates. *S. ionantha* “Aloha Orchid” genotype has the highest percentage of viability (94.32%) and germination capacity (45.73%) and a rich content of carbohydrates and polygalacturonic acids. In *S. rupicola* and *S. ionantha* genotypes, the rich content of polygalacturonic acids, lipids, and carbohydrates favourably influenced the germination capacity. Spectroscopic result indicates, through different absorbance band intensity, a possible link between biochemical composition, viability, and germination capacity of *Saintpaulia* pollen. To determine exactly the relation between biochemistry and biological processes, it is necessary to initiate quantitative researches.

1. Introduction

The *Saintpaulia* (H. Wendl.) genus has an important economical and ornamental value, both in Romania and in various European countries [1, 2]. This is reflected by the presence of 2000 existing cultivars [3] and the interest of breeders to create and obtain new cultivars with superior morphological characters. African violets are the most popular indoor plants [1, 4], preferred for their tolerance to north exposures, their relatively quick and easy propagation, their flowering throughout the year, and their permanent decor through their flowers and leaves [5].

African violet (*Saintpaulia* H. Wendl.) is native to Eastern Africa [6, 7], tropical Africa [8], Tanzania, and Kenya [3]. It is

an angiosperm of the order Lamiales, in the family Gesneriaceae [2, 9]. Much data exists on vegetative propagation of *Saintpaulia* genotypes [1, 2, 7, 10, 11] but only a few studies refer to their palynological analysis.

In order to identify chemical compounds of different biological materials such pollen [12, 13], FT-IR spectroscopy is one of the most widely used methods. According to dedicate literature [14, 15], this analytical process was used to identify [16, 17], determine, classify, discriminate, and characterize [18, 19] pollen of many ornamental plants. The relative biochemical composition using FT-IR [20], germinability, and pollen viability has been studied in various ornamental plants [21–25], including different species from the conifers,

TABLE 1: The morphology of *Saintpaulia* genotypes.

Code	Genotype	Flower colour*	Brief description [4, 6]
S1	<i>S. ionantha</i> “Red Velvet”	Deep pink 52B	Single flower, medium green foliage
S2	<i>S. ionantha</i> “Jolly Red”	Strong red 53B	Single flower, light green foliage
S3	<i>S. ionantha</i> “Aloha Orchid”	Light purple RHS 77D	Semidouble flower, medium green foliage
S4	<i>S. ionantha</i> “Hot Pink Bell”	Light pink 39D	Single flower, dark green foliage
S5	<i>S. ionantha</i> “Park Avenue Blue”	Signal violet RHS 77D and greenish white 155C	Single flower, dark blue with a white eye on the middle of corolla, medium green foliage
S6	<i>S. ionantha</i> “Lucky Ladybug”	Strong red 53B	Single flower, dark green, quilted foliage
S7	<i>S. ionantha</i> “Crimson Ice”	Light pink 39D	Single flower, white with pink thumbprint on each petal, and dark green foliage
S8	<i>S. ionantha</i> H. Wendl.	Strong violet RHS 90B	Violet single flower, dark green foliage
S9	<i>S. ionantha</i> “White Queen”	Greenish white 155C	Double flower, light green foliage
S10	<i>S. ionantha</i> “Painted Silk”	Strong violet RHS 90B	Single flower, light green girl-type foliage
S11	<i>S. ionantha</i> “Pink Pussycat”	Light pink 39D	Single flower, dark green foliage
S12	<i>S. ionantha</i> “Buffalo Hunt”	Strong red 39D	Semidouble flowers, with a slightly ruffled edge; foliage is dark green
S13	<i>S. rupicola</i> B. L. Burt	Strong violet RHS 90B	Double flower, long stalk, and light green foliage
S14	<i>S. ionantha</i> “Tomahawks”	Strong red 39D	Semidouble, bright red, dark green foliage
S15	<i>S. grotei</i> Engl.	Light blue RHS 104D	Single small flowers, light green foliage

*The colours of genotypes were established according to the RHS colour chart.

monocotyledons, eudicots, and magnoliids [16]; however these studies do not include the *Saintpaulia* genus.

This paper is a comprehensive study of pollen belonging to 15 African violet genotypes. The lack of consistent and relevant data on bioactive elements of *Saintpaulia* pollen and also their connection on the viability and germination make this a pioneer study, and the results obtained allow a selection of valuable genotypes for breeding programs.

2. Material and Methods

2.1. Plant Material. In order to investigate the chemical composition, viability, and germination capacity, pollen was collected in October from the mature anthers of 15 genotypes of *Saintpaulia ionantha* H. Wendl. (Table 1). The plants were cultivated under the same environmental conditions (84% average air humidity and 23°C average temperature) at the didactic greenhouse of the Department of Floriculture within the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (UASMVCN).

2.2. Biochemical Composition. The research of spectral differentiation in pollen grain biochemistry was conducted in the Raman and IR Spectrometry Laboratory, at the UASMVCN, with the FT/IR-4100 spectrometer (Jasco Analytical Instruments, Easton, USA), with a spectral region between 4000 and 350 cm⁻¹ and a resolution of 4 cm⁻¹. The pellet mode was chosen, in which the pollen samples (3 mg) were mixed with 200 mg of potassium bromide and then compressed into tablets (Specac, IR accessory for producing pills) [13]. For each experimental sample (S1 ··· S15), 264 scans were made and one final IR spectrum was illustrated in Figures 1–3.

The Spectra Manager software package was used for scanning samples. The data were processed using ORIGIN 8.5 Pro software.

2.3. Pollen Viability. In order to determine the viability test, the collected anthers were put in a Carnoy solution for 2 hours, after which they were washed in 80% ethyl alcohol. The determination of pollen viability was obtained by staining with potassium iodide (25%). Pollen viability was obtained by staining with potassium iodide (25%). The brown pollen was considered viable, and the colourless one unviable [26–28]. The pollen quantification was made by observing the pollen in ten fields in each of five replications using Aigo Digital Microscope EV5610.

2.4. Pollen Germination. The germination and pollen viability were evaluated in accordance with the methodologies described by Gudadhe and Dhoran [23], Bodhipadma et al. [29], and Cordea [28]. The germination of pollen was performed on solid nutrient medium (15% sucrose, 85% humidity, and 22°C temperature), and their counting was done using an electronic microscope (Aigo Digital Microscope EV5610, Beijing Research Institute of Precision Instrument Aigo Co., Ltd.). Counts were made in ten fields in each of five replications. The total number of grains on the field and the number of the germinated and the ungerminated pollens were recorded [27, 28].

Data on viability and germination of *Saintpaulia* pollen were analysed using the ANOVA test, where significant differences between mean values were separated using the Duncan’s test.

3. Results and Discussions

3.1. The Biochemical Composition of *Saintpaulia* Pollen. Fourier transform infrared spectroscopy (FT-IR) is modern analytical method, allowing rapid examination of the relative biochemical compositions of pollen or other biological material [12, 13]. The biomolecular constituents of pollen, which can be

TABLE 2: The spectral bands assignment using the FT-IR method, in the case of *Saintpaulia* genotypes.

Spectral zone	Peak frequency cm^{-1}	Chemical bonds
1,800–1,500 cm^{-1}	$\approx 1,730 \text{ cm}^{-1}$	C=O stretch (lipids, triglycerides, and alkyl-esters)
	1,660–1,666 cm^{-1}	C=O stretch (amide I-proteins)
	1,608 cm^{-1}	COO ⁻ antisymmetric stretch (acidic group of polygalacturonic acids)
	1,540–1,550 cm^{-1}	N–H deformation; C–N stretch (amide II, proteins and lignin)
	$\approx 1,514 \text{ cm}^{-1}$	C=C–C (approximation of aromatic ring bonding) (sporopollenin)
1,400–1,000 cm^{-1}	1,416–1,418 cm^{-1}	COO ⁻ symmetric stretch (acidic group of polygalacturonic acids)
	1,440–1,450 cm^{-1}	CH ₂ , CH ₃ deformation (acidic group of polygalacturonic acids)
	1,318–1,321 cm^{-1}	(C–C, C–O) (acetylenic compounds)
	1,255–1,257 cm^{-1}	C–O–H deformation; C=O stretching of phenolics (lipids and triglycerides, pectins, and carbohydrate molecule)
	1,104–1,106 cm^{-1}	C–OH skeletal; C–O–C sugar ring (carbohydrate molecule)
900–800 cm^{-1}	1,050–1,055 cm^{-1}	C–O–C stretch; C–OH stretch; C–OH deformation; C–O–C deformation, pyranose, and furanose ring (carbohydrate molecule)
	$\approx 920 \text{ cm}^{-1}$	C–H (β -glycosidic bond) (carbohydrate molecule)
	830–836 cm^{-1}	C–O–C (α -glycosidic bond) (carbohydrate molecule)

* See assignment in text.

identified by this method, are lipids, proteins, carbohydrates, and sporopollenin. These are the main structural and nutritional elements of pollen responsible for the majority of the phenotypical, physiological, and biochemical manifestations [16, 30].

Previous studies have shown that there are significant differences at the level of the biochemical composition of pollen belonging to related species; however there are few studies on the biochemical differences of pollen between genotypes of the same genus [18]. For the 15 samples of *Saintpaulia* pollen, it was established that, in order to describe the molecular vibrations, the area of analysis of the spectral region is 1,800–800 cm^{-1} (Table 2, Figures 1–3). The genotypes were arranged in three groups: I (S1, S2, S6, S12, and S14), II (S3, S8, S10, S13, and S15), and III (S4, S5, S7, S9, and S11) based on their colours.

For the first group of plants, the zone between 1,800 and 1,500 cm^{-1} is characterized by the presence of a band with a value around 1,730 cm^{-1} (S1, S12, and S14) and two bands for the 1,660–1,500 cm^{-1} zone (Figure 1). The band at 1730 cm^{-1} (C=O stretch) indicates the presence of lipids, triglycerides, and alkyl-esters, according to Yang and Yen [31] and Mularczyk-Oliwa et al. [12]. Proteins are characterized by a strong band at 1,666 cm^{-1} (amide I: C=O stretch) in sample S1 [12, 16]. Notably, the pollen grains of this genotype have rich protein content, the most important nutritional indicator of pollen [30]. The presence of aromatic rings from sporopollenin is also observed, in the same spectral area, by the appearance of the absorption band of 1,514 cm^{-1} , described by Zimmermann and Kohler [18] (Table 2, Figure 1).

In the spectral region of 1,500–900 cm^{-1} a prominent band around the value of 1,400 cm^{-1} (COO⁻ stretch and CH₂ and CH₃ deformation) appeared, which is attributed to the

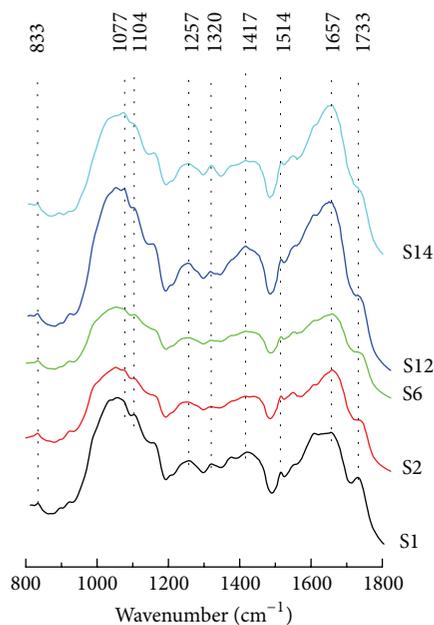


FIGURE 1: The FT-IR absorption bands of the spectral region 1,800–800 cm^{-1} (4 cm^{-1} resolution) of *Saintpaulia* genotypes pollen for the plants of the first group.

presence of lipids and triglycerides, observed by Mularczyk-Oliwa et al. [12] and Zimmermann and Kohler [16]. The peak of 1,318 cm^{-1} is attributed to the presence of acetylenic compounds [12, 32]. The signal at 1,255 cm^{-1} is associated with carbohydrates molecules [31, 33]. The most intense band in this spectral region is at 1,106 cm^{-1} (C–OH skeletal; C–O–C)

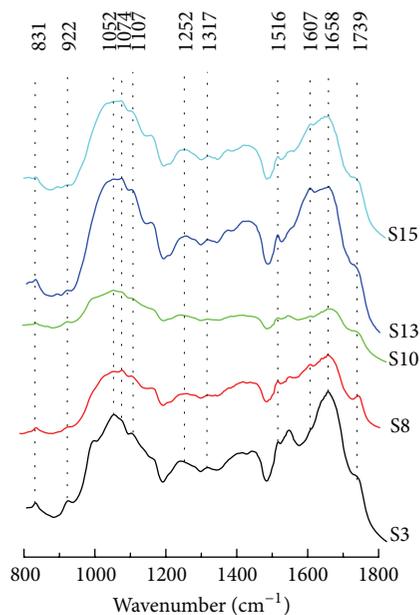


FIGURE 2: The FT-IR spectral bands assignment in the 1,800–800 cm^{-1} zone (4 cm^{-1} resolution) in the *Saintpaulia* genotypes of pollen from the second group of plants.

in S6 and S12 samples [12]. Carbohydrate molecules can also be observed, around the band of 830 cm^{-1} (C–O–C).

For the *second group of plants* (Figure 2), a strong vibration was observed around the value of 1,740 cm^{-1} . This vibration is better defined in the S8 genotype moving unvaryingly to 1,732 cm^{-1} (S15). The presence of amide I was noticed through C=O stretch band around 1,660 cm^{-1} , as illustrated by several authors [12, 18, 34].

The spectral band at 1,608 cm^{-1} (COO^- antisymmetric stretch) was more visible in S8 and S15 genotypes, while in S3 it appears as a shoulder and can be associated with the vibration of polygalacturonic acids. The absorption band of 1,517 cm^{-1} was found in all genotypes from this group of plants, being correlated to the presence of aromatic rings from sporopollenin [18].

Genotypes S13 and S15 presented strong vibrations around the value of 1,318 cm^{-1} (C–O skeletal), attributed to the existence of acetylenic compounds, as reported by Coates [32] and Mularczyk-Oliwa et al. [12]. The vibrations around 1,241 cm^{-1} and 1,255 cm^{-1} (C–O stretch) are generated by lipids and triglycerides [19]. In the spectral region of 1,100 cm^{-1} , the most well-defined vibrational bands are located at 1,055 cm^{-1} (S3 genotype). These bands unvaryingly moved towards 1,074 cm^{-1} in S8 and S10 and 1,077 cm^{-1} for S13 and S15, attributed to the carbohydrates content according to Zimmermann and Kohler [16]. Stronger vibrations were observed in S8, S13, and S15, which represent three important species in breeding (*S. grottei*, *S. ionantha*, and *S. rupicola*). Carbohydrates molecules are associated with values 922 cm^{-1} and 830 cm^{-1} (C–O–C).

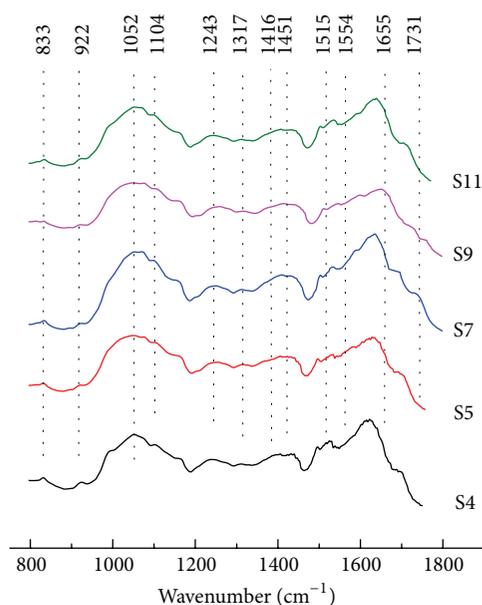


FIGURE 3: The molecular vibrations in pollen samples at 1,800–800 cm^{-1} region (4 cm^{-1} resolution) in *Saintpaulia* genotypes from the third group of plants.

In the *third group of plants*, for S4 and S5, there were two strong bands in the 1,550 cm^{-1} region, which are characterized by the presence of proteins (amide II: N–H deformation, C–N stretch) as indicated by Mularczyk-Oliwa et al. [12] and Zimmermann and Kohler [18] (Figure 3).

The presence of aromatic rings in sporopollenin is visibly delimited around the value of 1,515 cm^{-1} in all genotypes [16]. Two peaks appeared in S4 and S5 genotypes at 1,416 cm^{-1} (COO^- symmetric stretch) and 1,450 cm^{-1} (CH_2 , CH_3 deformations), the result of the presence of polygalacturonic acids. The presence of the signal at 1,257 cm^{-1} is usually associated with lipids, triglycerides, and carbohydrates molecules. The absorption band between 1,318 and 1,321 cm^{-1} (C–C, C–O) is associated with the existence of acetylenic compounds [12, 32].

The carbohydrate band is presented around 1,055–836 cm^{-1} through C–O–C stretch, C–OH stretch, C–OH deformation, C–O–C deformation, pyranose and furanose ring, and α -glycosidic and β -glycosidic vibrations, confirmed by Mularczyk-Oliwa et al. [12] and Zimmermann and Kohler [18].

The most important bioactive elements were present in all the 15 *Saintpaulia* genotypes analyzed, but the spectral intensity between their characteristic bands substantially varies as demonstrated by Zimmermann and Kohler [16] and confirmed by Žilić et al. [30].

3.2. Pollen Viability of *Saintpaulia* Genotypes. Pollen quality analyzed through the viability perspective and germination capacity can offer important information for breeders,

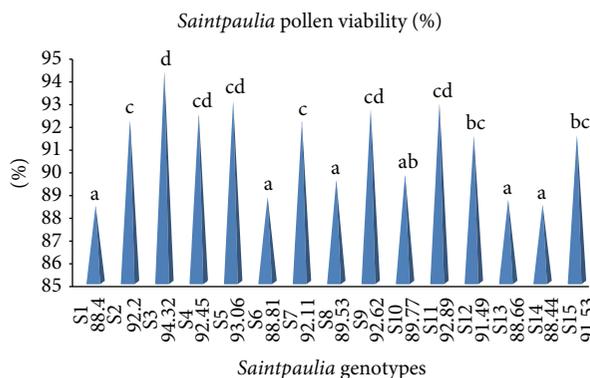


FIGURE 4: Percentage of pollen viability in 15 genotypes of *Saintpaulia* using the potassium iodide stain method (25%). *Values are means of 5 replications \pm SD (1.77–2.05). Small letters represent the statistical significant differences at $P < 0.05$ (Duncan's test).

geneticists, and growers [34, 35]. According to Rodriguez-Riano and Dafni [36] and Firmage and Dafni [37], the pollen viability depends on the staining method and on species.

The highest percentage of viability was recorded in S3 (94.32%), distinguishing itself by the rich content of carbohydrates (Figures 2 and 4). The same high viability was recorded in S4, S5, S9, and S11 genotypes, not differing statistically from S3. The lowest viability percentage was recorded in S1 (88.40%), even if the pollen grain of this genotype has a high content of proteins. Genotypes S6, S8, S10, S13, and S14 had percentage of viability similar to that in S1 (Figure 4).

3.3. Pollen Germination of *Saintpaulia* Genotypes. For evaluation of the maximum germination potential and the proper development of the pollen tube, the most quick, simple, and complete method is *in vitro* demonstrated and confirmed by Fei and Nelson [38], De Assis Sinimbú Neto et al. [39], Ahmad et al. [40], Gudadhe and Dhoran [23], Bodhipadma et al. [24], and Soares et al. [25].

Several studies show that this process can be influenced by exogenous factors, composition of the germination environment, and the harvesting conditions [25, 30, 41–44], but at the same time this can be a genotypic characteristic.

The highest germination percentage was recorded in S8 (74.16%), followed by S15 (65.46%) and S4 (56.46%). This can be correlated with the high lipid content, an essential element in the storage of nutrients and energy necessary for germination. Lipid bodies are used as cytological markers so as to monitor the vital processes of pollen grains [41]. The results interpreted by means of the Duncan's test ($P < 0.05$) show that significant differences are recorded in S12 (9.17%), S7 (29.51%), S3 (45.73%), S4 (56.46%), S15 (65.47%), and S8 (74.16%) genotypes (Figure 5).

The *Saintpaulia* pollen germination process differed from one genotype to another. The above results show that some genotypes (Figure 5) do not reach the minimum germination level (30%), which means that these genotypes are recommended only as maternal genitors in breeding, even if these have high pollen viability [28, 45]. Very good results

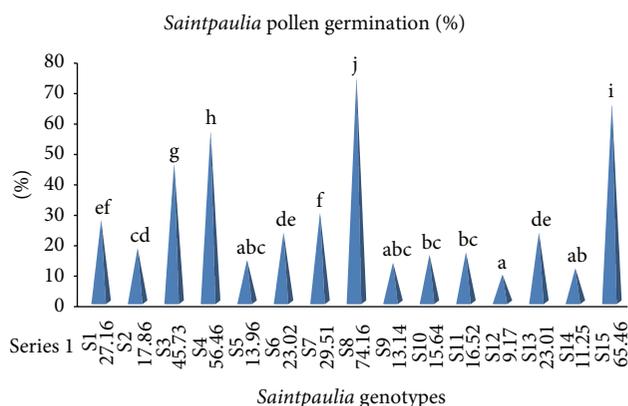


FIGURE 5: The germination percentage of the pollen grains in *Saintpaulia* genotypes on solid medium (15% sucrose, 85% moisture, and 22°C temperature). *Values are means of 5 replications \pm SD (5.51–6.38). Small letters represent the statistical significant differences at $P < 0.05$ (Duncan's test).

regarding the viability were obtained in S3, S4, S5, S9, and S11 genotypes, which also have a rich content of polygalacturonic acids, lipids, and carbohydrates. Genotypes S8, S15, and S4 are noted for high germination capacity. Due to these qualities, the above mentioned genotypes can be used in hybridization programs as suggested by Kolehmainen and Mutikainen [3].

4. Conclusions

The IR spectroscopy results show that the structural and nutritional elements of pollen are present in all analyzed genotypes. The detailed interpretation of spectral bands shows that the spectral intensity of various bioactive components substantially differs from one genotype to another within the same genus. The data obtained in this study allow for the rapid expansion of the standardized FT-IR spectra and can serve as a starting point for the identification, classification, and biochemical characterization of pollen, results confirmed by Zimmermann and Kohler [16].

Genotypes S3, S4, S5, S8, S9, and S11 can be recommended as potential genitors in breeding for viability and germination purposes. The genotypic behaviour in viability and germination process probably depends on the biochemical components and the connection between them. Research in this sector should continue so as to better highlight the contribution of each element in the evolution of these biological processes, by obtaining quantitative data.

The results obtained based on the research and investigations are important in the global research programs which aim to build and introduce new genotypes of ornamental plants to be competitive in the international ornamental's assortment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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