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Pollen Morphology and Variability of *Abies alba* Mill. Genotypes from South-Western Poland

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Abstract: The objective of this study was to investigate pollen morphology and ranges of intraspecific variability of *Abies alba* Mill. Pollen grains were collected from nine clonal seed orchards of *A. alba* in the Sudety Mountains, (South-Western Poland). At each seed orchard, 4–6 grafts were selected. Each individual (graft) was represented by 30 pollen grains and 1440 pollen grains were measured totally. Eight quantitative and four qualitative features of pollen grains were analysed. The diagnostic features of pollen grains for the studied species were: Exine surface of pollen corpus (cappa and leptoma) and sacci, the length of the polar axis (P), pollen shape (P/E ratio), and a new trait—saccus shape (A/B ratio — saccus width (A) to his length (B)). Pollen features made possible to differentiate seven individual genotypes (samples). To our knowledge, this is the first time that the intraspecific and interindividual variability of pollen grains of *A. alba* were investigated. The most different were the pollen grains from samples—genotypes 13 (Bystrzyca Kłodzka) and 18 (Jugów), and also (although to a lesser extent) genotypes—11 (Kamienna Góra), 30, 31 (Jugów), and 44 (Szklarska Poręba). No significant relationships were observed between the pollen grain traits and the geographical location of the collection sites.

Keywords: pollen morphology; intraspecific variability; DNA variability; genotype; *abies*; *abies alba*

1. Introduction

Biologically, the production of pollen is a crucial stage of sexual reproduction in plants, involving the transfer of parental genes to offspring generations. However, from a forestry perspective pollen production is the process that affects the amount and frequency of seed crops in economically important trees. By simplifying reproduction to the perspective of pollen donors only, the seed crop to a large extent depends on the pollen amount, its quality, and efficiency of the dispersal. In wind-pollinated plants, a majority being the European forest tree species, stochasticity of the pollination process evolutionarily has required the production of a large amount of pollen [1]. Pollen dispersal distance affects pollination rate and thus seed crop is largely the function of intrinsic pollen grain characteristics (size, weight, and morphology) that governs the physics of dispersion. The size of pollen production, the dispersal distance, and pollen viability are all modified under environmental influence [2], which attains a special context under the observed climate transformation.

Among several species from the genus *Abies* present in Europe, *A. alba* Mill. possesses the widest occurrence spanning from the mountainous areas in Southern Europe (Spain) up to the lowlands of Central Europe (Poland). It is an ecologically and economically important forest tree species and one of the most productive among native European conifers [3]. However, due to the peculiar forest management requirements of the species that reflect its late-successional character, other conifers have been favoured over silver fir in the forestry since the 19th century [4]. In many areas of Central Europe that are suitable for silver fir, the species has been replaced by *Picea abies* (L.) Karst. or *Fagus sylvatica* L. to cover the great demand for wood at certain times [5,6]. However, both of the above-mentioned species are more drought sensitive in comparison to silver fir [4,7,8]. Considering the results that suggest a great potential of silver fir in view of the expected climate changes, it seems to be the species of choice for European forestry, especially in the context of the dramatic conditions of the Norway spruce stands to be decimated with bark beetle [4] and there is a retreating trend predicted for Scots pine [9]. Currently, the share of silver fir in Poland is 3.2% of forest areas [10]. Populations are mostly distributed in the mountainous areas, while the lowland range is highly fragmented and low-density stands prevail [11].

Mast years in silver fir occur every three to five years but these crops often display a high proportion of empty seeds [12], which may be related to its lower dispersal ability of pollen. It is because, *A. alba* produces very heavy pollen of ca. 251.1×10^{-6} g per 1000 grains and a sedimentation velocity of ca. 0.12 m s^{-1} [13]. These features may likely reduce the potential of pollen migration and affect successful pollination and finally, seed production, especially in the complex mountainous landscape or fragmented populations. Experimental data obtained from the parentage analysis based on the genetic markers indicate that pollen movement in old-grown silver fir stands varies substantially. The pollen can be dispersed on average over a distance of ca. 100 m in populations from the northern range margins (Poland) [14,15]. However, a very local dispersion was noted reaching only 11 m on average in old populations from the southern range margin (France) [14]. In the years with low flower production or in isolated, fragmented, and low-density stands, self-fertilization may be common (ca. 95%) [16]. It likely reflects the high inbreeding depression, typical for many conifers [17].

The knowledge of the morphological structure of *Abies* Mill. pollen grains is incomplete due to the small amount of research on this subject, especially in Europe. Researchers usually limit their analyses to individual and/or the most important pollen grain features, mainly pollen size and shape, or exine ornamentation. As yet no other research on the intraspecific variability of *A. alba* pollen has been undertaken. Ting [18] studied the pollen morphology of the five *Abies* species (*A. bracleafa* (D. Don) Nutt., *A. concolor* (Gordon et Glend.) Lindl. ex Hildebr., *A. grandis* (Douglas ex D. Don) Lindl., *A. magnifica* Murr., and *A. procera* Rehder) from California. Gudeski [19] examined and compared pollen grains of *A. alba* from populations in Macedonia and of *A. cephalonica* Loudon from Parnis in Greece. Bagnell Jr. [20] showed that three species of *Abies* (*A. amabilis* Douglas ex J. Forbes, *A. grandis*, and *A. lasiocarpa* (Hook.) Nutt.) differ in the respect of the morphology of the impression mark. Dobrinov and Gagov [21] analysed the morphology, viability, and storage of pollen from four native populations of *A. alba* in Bulgaria. The "Pal dat" database, established by Halbritter [22–24], contains brief descriptions of pollen of *A. cephalonica*, *A. concolor*, and *A. nordmanniana* (Steven) Spach. Khan et al. [25] described two species from Pakistan—*A. pindrow* (Royle) Spach. and *A. spectabilis* (D. Don) Mirb. According to the palynological studies mentioned, species from the genus *Abies* produce heteropolar monads. Pollen grains are large or very large, bisaccate (rarely one or trisaccate) with the impression mark on the proximal face. They have one aperture, the leptoma. The exine surface is psilate with or without perforations, regulate, or verrucate [18–20,22–26].

The main aim of this study was to supplement knowledge concerning pollen morphology in *A. alba*, an important forest tree species in Europe. Particularly, we aimed at establishing the pollen morphology and variability of *A. alba* collected in nine provenance areas located in South-Western Poland. The aims of this study were to verify the usefulness of the previously described as well as the new quantitative and qualitative pollen features and to describe, for the first time, the intraspecific and

inter-individual variability of pollen of the studied *A. alba* genotypes. The obtained results will be useful in future studies on the reproduction of *A. alba* and contribute to its restitution in Poland.

2. Material and Methods

2.1. Sampling and Genotyping

The analysis was conducted on 48 genotypes (samples) of *A. alba* that grow in 9 native localities of the studied species in the Sudetes Mountains located in South-Western Poland. The clonal seed orchards from which pollen was collected, were established for the 'Program of the silver fir *Abies alba* Mill. restitution in the Sudety Mountains' and the clone archive in the Karkonosze National Park. A list of the studied samples is shown in Table 1. As the pollen samples were taken from trees growing in the clonal seed orchards (Figure 1), there was a potential risk of errors in the labelling that would lead to the sampling of the same genotype, i.e., grafts within the same clone. To ensure that pollen analysis includes distinct individuals, genotyping with 6 nuclear microsatellite markers was done. Needle samples were taken from each individual tree subjected to pollen morphology analysis. Genomic DNA was extracted using the protocol of [27]. For the purpose of genotyping, a set of 6 nuclear microsatellites was used as follows: SFb5, SF333, SF1, SF239, SF78, and SFb4 [28,29]. Loci were amplified in a single PCR-multiplex reaction with the Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with ca. 20 ng of genomic DNA. The thermal profile included an initial denaturation at 95 °C for 15 min., and 10 touchdown cycles as follows: 94 °C for 30 s, 60 °C for 30 s (−1 °C per cycle), and 72 °C for 40 s, and 30 more cycles as follows 94 °C for 30 s, 50 °C for 50 s, 72 °C for 40 s, and a final extension of 7 min at 72 °C. Analysis of the fluorescently labelled PCR products was done on an ABI PRISM 3130 genetic analyser with the GeneScan 500-LIZ size standard (Life Technologies, Bleiswijk, The Netherlands). Genotypes were scored using the GeneMapper v. 4.0. software package (Applied Biosystems, Foster City, CA, USA).

Table 1. List of localities of the studied *Abies alba* genotypes.

Sample Numer	Clone Numer	Seed Orchard Location	Abbreviation	Geographical Coordinates (N/E)
1	40,077	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309
2	4064	Bystrzyca Kłodzka Forest District Pokrzywno	B—Bystrzyca	50.3742 16.50961
3	4144	Bystrzyca Kłodzka Forest District Pokrzywno	B—Bystrzyca	50.3742 16.50961
4	20,011	Karkonosze National Park Karpacz	K—Karpacz	50.76456 15.68285
5	10,041	Karkonosze National Park Karpacz	K—Karpacz	50.76456 15.68285
6	40,019	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309
7	40,036	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309
8	40,056	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309
9	87	Kamienna Góra Forest District Ogorzelec	KG—Kamienna Góra	50.76653 15.8967
10	142	Kamienna Góra Forest District Ogorzelec	KG—Kamienna Góra	50.76653 15.8967

Table 1. *Cont.*

Sample Numer	Clone Numer	Seed Orchard Location	Abbreviation	Geographical Coordinates (N/E)
11	127	Kamienna Góra Forest District Ogorzelec	KG—Kamienna Góra	50.76653 15.8967
12	24	Snieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
13	4063	Bystrzyca Kłodzka Forest District Pokrzywno	B—Bystrzyca	50.3742 16.50961
14	4002	Bystrzyca Kłodzka Forest District Pokrzywno	B—Bystrzyca	50.3742 16.50961
15	60,046	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205
16	3021	Lądek Zdrój Forest District Trzebieszowice	LZ—Lądek Zdrój	50.34253 16.77332
17	198	Śnieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
18	5341	Jugów Forest District Wojbórz	JUG—Jugów	50.5089 16.63274
19	5335	Jugów Forest District Wojbórz	JUG—Jugów	50.5089 16.63274
20	12	Śnieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
21	235	Śnieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
22	6031	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
23	6078	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
24	60,017	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205
25	6074	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
26	3014	Lądek Zdrój Forest District Trzebieszowice	LZ—Lądek Zdrój	50.34253 16.77332
27	3158	Lądek Zdrój Forest District Trzebieszowice	LZ—Lądek Zdrój	50.34253 16.77332
28	40,073	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309
29	60,135	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205
30	5347	Jugów Forest District Wojbórz	JUG—Jugów	50.5089 16.63274
31	5262	Jugów Forest District Wojbórz	JUG—Jugów	50.5089 16.63274
32	10,038	Karkonosze National Park Karpacz	K—Karkonosze	50.76456 15.68285
33	78	Śnieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
34	60,002	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205
35	30,006	Karkonosze National Park Karpacz	K—Karkonosze	50.76456 15.68285
36	20,019	Karkonosze National Park Karpacz	K—Karkonosze	50.76456 15.68285
37	4122	Bystrzyca Kłodzka Forest District Pokrzywno	B—Bystrzyca	50.3742 16.50961
38	40,049	Karkonosze National Park Jagniątków	JAG—Jagniątków	50.82693 15.63309

Table 1. *Cont.*

Sample Numer	Clone Numer	Seed Orchard Location	Abbreviation	Geographical Coordinates (N/E)
39	65	Kamienna Góra Forest District Ogorzelec	KG—Kamienna Góra	50.76653 15.8967
40	15	Kamienna Góra Forest District Ogorzelec	KG—Kamienna Góra	50.76653 15.8967
41	133	Śnieżka Forest District Maciejowa	M—Maciejowa	50.91411 15.82753
42	6056	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
43	5254	Jugów Forest District Wojbórz	JUG—Jugów	50.5089 16.63274
44	60,086	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205
45	6094	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
46	5314	Zdroje Forest District Duszniki	Z—Zdroje	50.40094 16.39554
47	3125	Lądek Zdrój Forest District Trzebieszowice	LZ—Lądek Zdrój	50.34253 16.77332
48	60,206	Karkonosze National Park Szklarska Poręba	SP—Szklarska Poręba	50.81837 15.47205

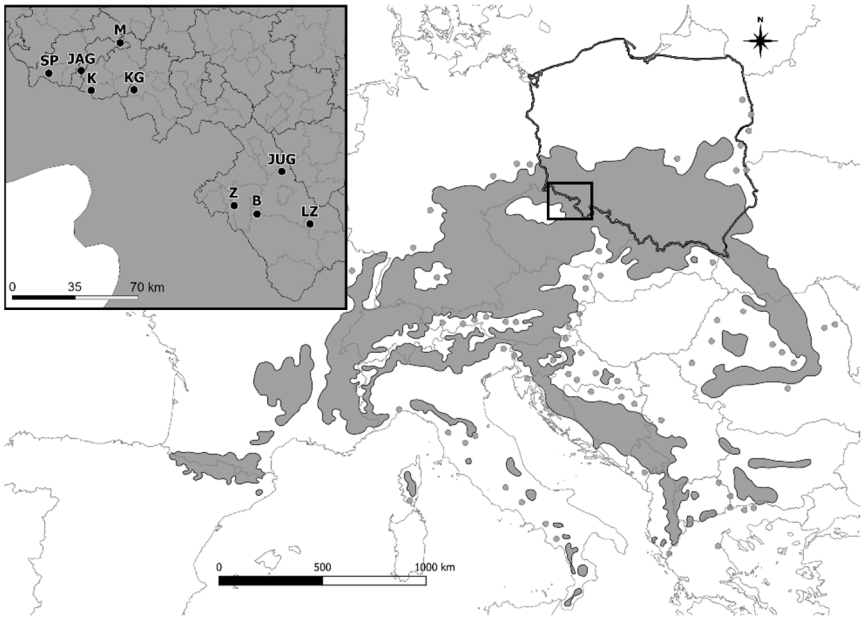

Figure 1. Distribution of *Abies alba* (source Euforgen [30]) and location of the seed orchards.

2.2. Palynological Analysis

Pollen was collected at the end of April 2019 from the selected grafts and pooled into a single sample representing the genotype. The plant material was stored in the herbarium of the Department of Forest Botany at the Poznań University of Life Sciences (PZNF). In accordance with the study by Wrońska-Pilarek et al. [31], each sample consisted of 30 randomly selected, mature, and correctly formed pollen grains derived from a single genotype. In total, 1440 pollen grains were subjected to the analysis.

The pollen grains were prepared for light (LM) and scanning electron microscopy (SEM) using the standard acetolysis method described by Erdtman [32]. The collected pollen grains were placed in

tubes and then centrifuged with glacial acetic acid. Grains were mixed with the acetolysis solution, which consisted of 9 parts acetic anhydride and one part concentrated sulphuric acid. The mixture was then heated to boiling and kept in the water bath for 2–3 min. Samples were centrifuged in the acetolysis mixture, washed with acetic acid, and centrifuged again. The pollen grain samples were then mixed with 96% alcohol and centrifuged 4 times, with processed grains subsequently divided into two groups. One half of the processed sample was immersed in an alcohol-based solution of glycerin for LM, while the other was placed in 96% ethyl alcohol in preparation for scanning electron microscopy (SEM). Morphological observations were carried out using both a digital light microscope (Levenhuk D320L, Tampa, FL, USA) and a scanning electron microscope (Jeol 7001TTLS, Akishima, Japan). Pollen grains were measured in a polar distal view at a magnification of 640×. A total of 8 quantitative features of the pollen grains were analysed, i.e., the length of the polar axis (P) and equatorial diameter (E), the width of the base of saccus (A) and length of the saccus (B), the exine thickness (Ex), and P/E, Ex/P, and A/B ratios. The pollen shape classes (P/E ratio) were adopted according to the classification proposed by Erdtman [32]: Peroblate (less than 0.50), oblate (0.51–0.75), suboblate (0.76–0.88), oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), and subprolate (1.15–1.33). The following qualitative features were also analysed: The corpus and saccus outlines, pollen shape, and exine ornamentation.

The descriptive terminology follows Punt et al. [33] and Halbritter et al. [34].

2.3. Statistical Analysis

2.3.1. Genetic Analysis

The hierarchical analysis of molecular variance (AMOVA) with individuals nested within locations (orchards) was performed in GenAlEx v. 6.2 (Acton, Australia) to examine the distribution of variation and differential connectivity among individuals, locations, and individuals within locations. The significance of the diversity distribution was tested with 9999 permutations. Additionally, the level of genetic variability and genetic relationships among individuals and locations was further analysed with principal component analysis (PCA) also in GenAlEx. The differentiation between the sampled locations was tested with 1000 permutations.

2.3.2. Palynological Analysis

The normality of the distributions of the observed traits (P, E, A, B, Exp, P/E, Exp/P, and A/B) was tested using Shapiro–Wilk's normality test [35]. Multivariate analysis of variance (MANOVA) was performed for all 8 observed traits jointly. Next, one-way analysis of variance (ANOVA) was carried out to determine the effects of *Abies alba* genotypes on the variability of P, E, A, B, Exp, P/E, Exp/P, and A/B. The range values (minimal and maximal), mean values, and coefficients of variation of traits were calculated for all genotypes. Fisher's least significant differences (LSDs) were calculated for individual traits and on this basis, homogeneous groups of genotypes were determined. The relationships between observed traits were assessed on the basis of Pearson's correlation for means of genotypes. Relationships of observed traits were presented in the heatmap. Results were also analysed using multivariate methods. The canonical variate analysis was applied in order to present a multi-trait assessment of a similarity of tested genotypes in a lower number of dimensions with the least possible loss of information [36]. This makes it possible to illustrate the variation in genotypes in terms of all observed traits in the graphic form. Analysis of canonical variables is a statistical tool making it possible to solve the problem of multivariate relationships [37–40]. Mahalanobis' distance [41] was suggested as a measure of "polytrait" genotypes similarity [42], and its significance was verified by means of critical value D_{α} called "the least significant distance" [43]. Mahalanobis' distances were calculated for genotypes. The differences among analysed genotypes were verified by the cluster analysis using the nearest neighbour method and Euclidean distances. All the analyses were conducted using the GenStat 18th edition statistical software package.

3. Results

3.1. Genetic Variability

All used nuclear single sequence repeats (nSSRs) loci were polymorphic and complete genotypes for almost all individuals obtained (Appendix A, Table A1). Despite numerous repeats, the SF1 locus in the Maciejowa location did not give the amplification results. The SF78 locus was most polymorphic having 21 alleles, while the least polymorphic was SF1 with four alleles. According to AMOVA, the highest proportion of molecular variance was found within individuals (65%, $p < 0.05$), while among individuals it was 32% ($p < 0.05$), and only 3% concerned the inter-orchard level, but it was statistically significant ($p < 0.05$, Table 2). PCA conducted at the individual level showed an overlapping of genetic diversity ranges for individuals originating from different seed orchards (Figures 2 and 3). However, the analysis performed at the orchard level revealed a distinct character of three locations. The first principal coordinate (48.3% of total variation) showed the separate character of Lądek Zdrój, Karpacz, and Maciejowa, while the second one (38% of total variation) underlined a further distinction of the Maciejowa location.

Table 2. Analysis of molecular variances (AMOVA) based on nuclear single sequence repeats (nSSRs) in nine seed orchards.

Source	df	SS	MS	Est. var.	Percentage of Variation
Among Pops	8	29.481	3.685	0.071	3%
Among Indiv	39	114.217	2.929	0.720	32%
Within Indiv	48	71.500	1.490	1.490	65%
Total	95	215.198		2.280	100%

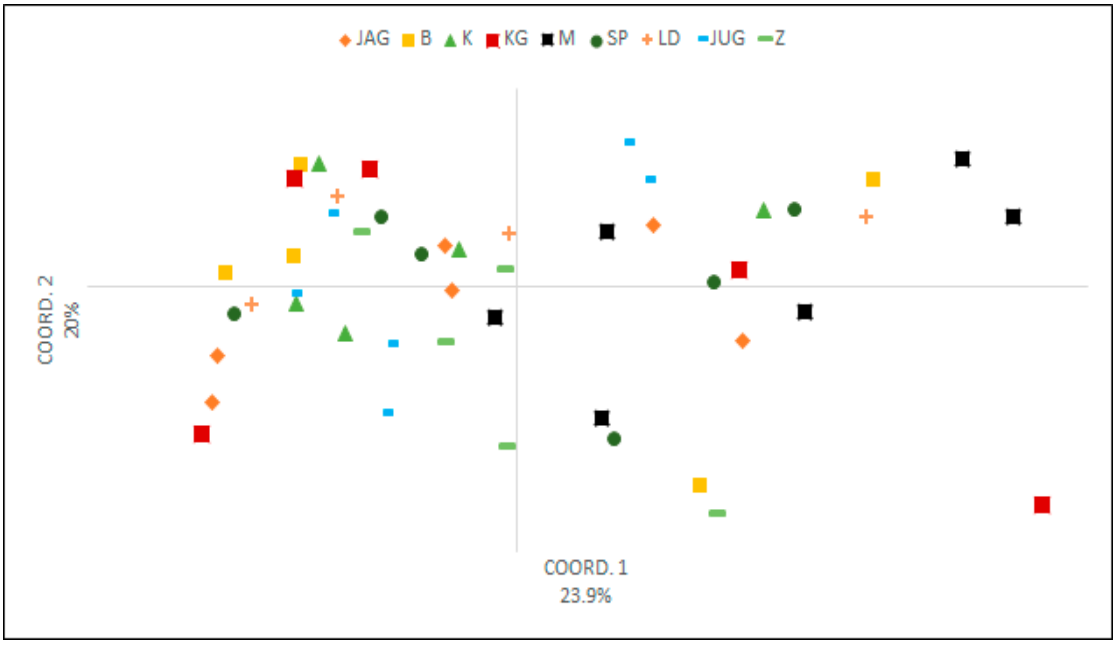

Figure 2. PCA on nSSRs conducted for 48 trees from nine seed orchards.

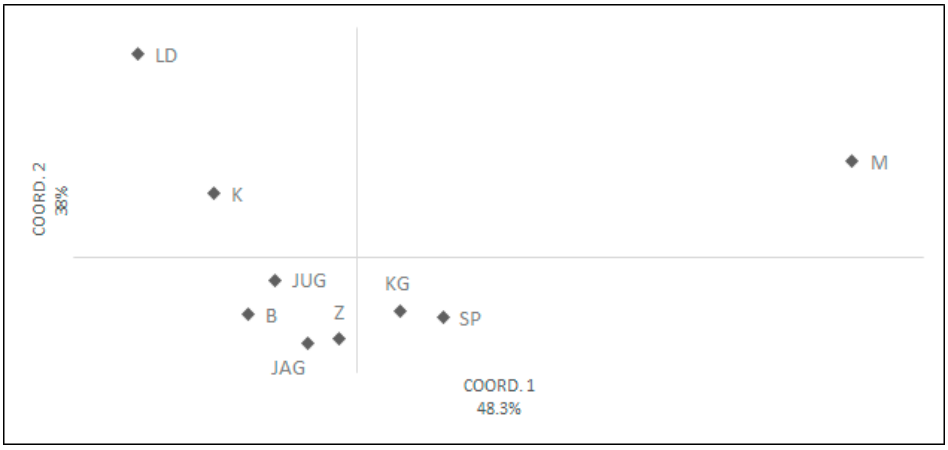

Figure 3. PCA on nSSRs conducted for seed orchards.

3.2. General Morphological Description of Pollen

Genotyping of the collected material with six microsatellites indicated that among the sampled individuals within each stand, distinct genotypes were present. Consequently, it assured that the analysis of pollen morphology involved distinct individuals. The genotypes of investigated individuals are given in the Appendix A.

A description of pollen grain morphology of the 48 genotypes (samples) of *Abies alba* under analysis is given below and illustrated in the SEM photographs (Figure 4A–F). The morphological observations for the quantitative features are summarised in Tables A2 and A3.

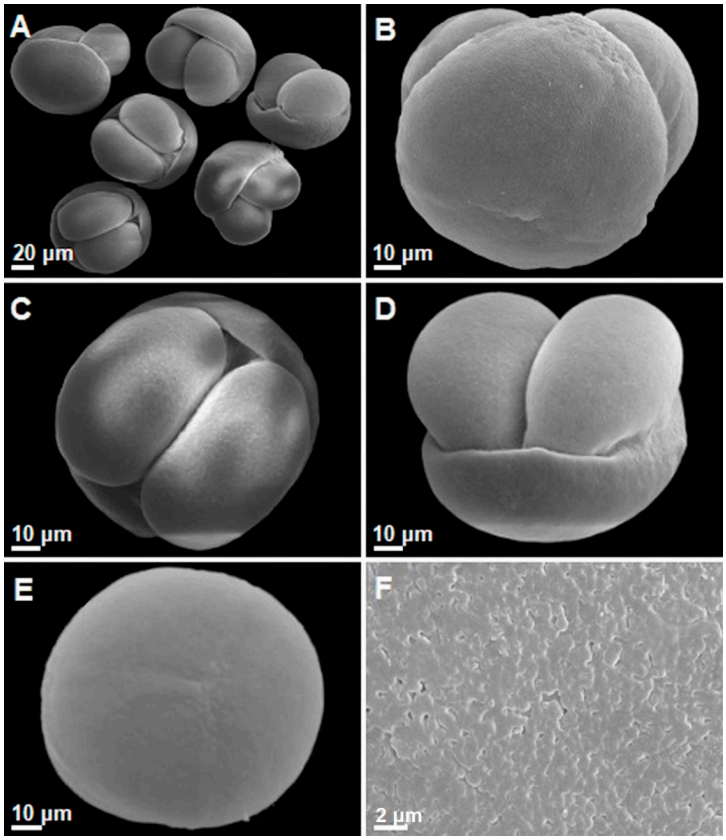

Figure 4. Pollen grains of *Abies alba*. (**A**)—group of pollen grains in the polar and equatorial view, (**B**)—polar proximal view, impression mark, (**C**)—two sacci in polar distal view, (**D**)—two sacci in equatorial view, (**E**)—polar proximal view, and (**F**)—psilate perforate exine surface.

The pollen grains of investigated *A. alba* genotypes were isodiametric, heteropolar monads (Figure 4A–E). They were bisaccate, with one aperture leptoma. On the proximal face of the pollen grain, Y-shaped impression marks were found to be more or less distinct.

According to Erdtman's [44] pollen size classification, most of the investigated pollen grains were large (97.36%) or very large (2.64%), with the polar axis (P) of the corpus (the central body of the pollen grains) ranging between 50.00 and 128.00 µm. The average value of P at the individual genotype level ranged from 73.10 to 92.50 µm. The widest range of this trait was observed in genotype 22 (50.00–128.00 µm) and the smallest in genotype 12 (62.00–84.00 µm). The shortest mean polar axis (P) was found in genotypes 12 and 13 (73.10 µm, 73.90 µm), while the longest was noted in genotypes 30 and 26 (90.30 µm, 92.50 µm) (Figure 5, Table A2).

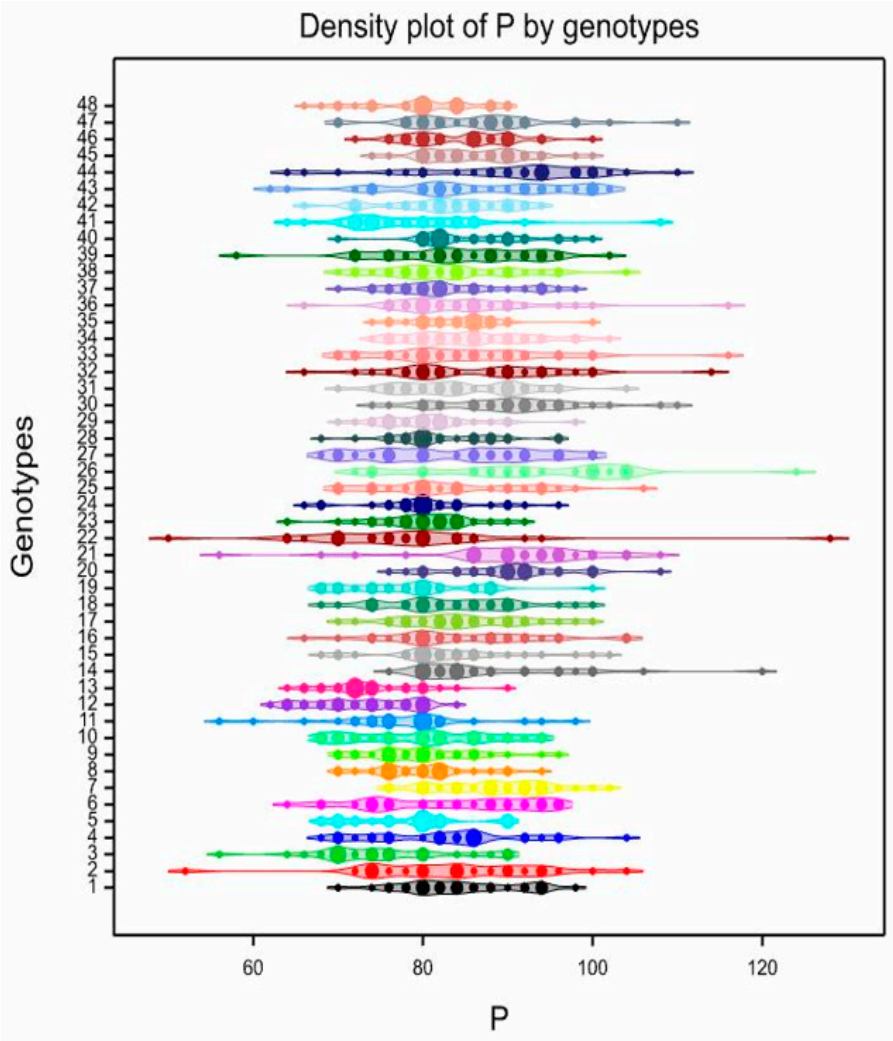

Figure 5. The density plot for the distribution of the shortest mean polar axis (P), classified by *Abies alba* genotypes. The observations are plotted along a line, with kernel density smoothed on either side to indicate the density of observations along the line. Numbers from 1 to 48, see Table 1.

Considering all the studied *A. alba* samples, the mean length of the equatorial diameter (E) of the pollen corpus was 100.29 µm, while the smallest value of this feature was 46.00 µm (genotype 4) and the largest amounted to 146.00 µm (genotype 44). The largest range of this trait was observed in genotype 4 (46.00–124.00 µm) and the smallest in genotype 12 (80.00–98.00 µm). The average value of E ranged from 82.90 to 113.90 µm (in genotypes 13 and 30) (Table A2, Figure 6).

The pollen corpus outlined in the polar distal, proximal, and equatorial views was mostly elliptic and rarely circular (Figure 4A–E).

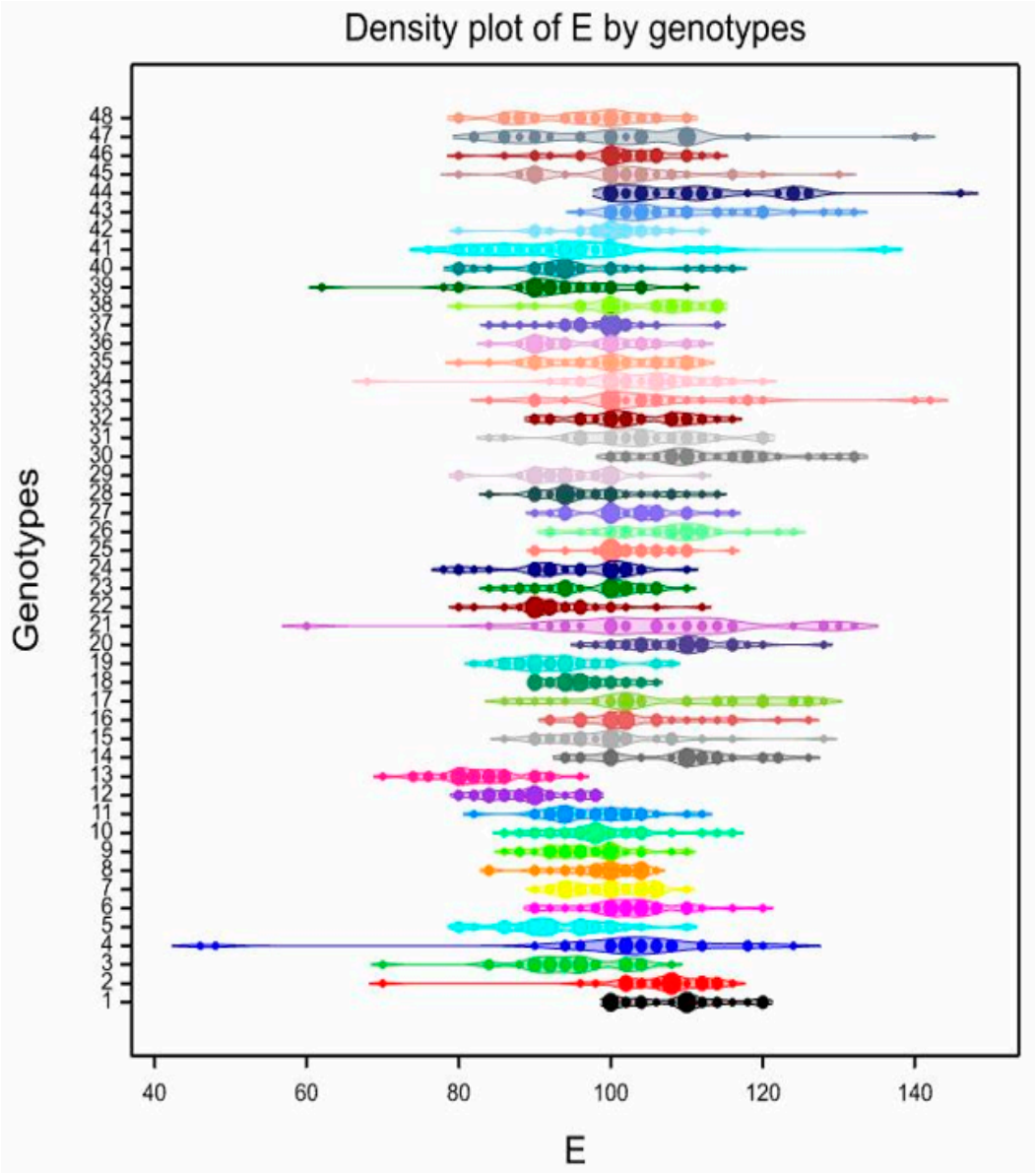

Figure 6. The density plot for the distribution of the mean length of the equatorial diameter (E), classified by *Abies alba* genotypes. Numbers from 1 to 48, see Table 1. Generally, the mean P/E ratio was 0.84 and ranged from 0.44 in genotype 44 to 1.83 in genotype 4. On average, the smallest value of the P/E ratio was in genotype 1 (0.78) and the largest in genotype 39 (0.91). The largest range of P/E ratio was found in genotype 4 (0.65–1.83) and the smallest one in genotype 28 (0.71–0.92) (Table 3, Figure 7).

In all the genotypes of *A. alba*, suboblate (50.83%), oblate-spheroidal (23.82%), and oblate (19.38%) pollen shape classes predominated. Rarely, prolate-spheroidal (3.40%) and spheroidal (1.94%) were noted. In seven pollen grains (0.49%) and two other (0.14%), subprolate and peroblate shapes were found, respectively.

Exine thickness of the corpus varied gradually. The thinnest was next to sacci in the leptoma area and the thickest in the cappa area. The mean exine thickness was 5.30 mm, with a range of 2.00 mm in 76 genotypes up to 18.00 mm in genotype 31. On average, the exine was the thinnest in genotype 11 (3.57 mm), while the thickest was found in genotype 18 (9.27 mm). In total, the relative thickness of the exine (Ex/P ratio) was on average 0.06 (ranging from 0.02 to 0.2) (Table 3).

Table 3. Minimal, maximal, and mean values as well as coefficient of variation (cv, in %) for Exp, P/E, Exp/P, and A/B. In columns, means followed by the same letters are not significantly different.

Genotype	Exp				P/E				Exp/P				A/B			
	Mean		Range	cv	Mean		Range	cv	Mean		Range	cv	Mean		Range	cv
1	4.10	klm	2–8	35.85	0.78	f	0.68–0.90	8.12	0.048	kl	0.02–0.08	33.88	2.16	defghi	1.39–3.57	27.22
2	4.67	ghijklm	2–6	23.42	0.79	def	0.67–0.96	10.66	0.057	fghijkl	0.02–0.12	31.03	2.43	abcdefgh	1.50–3.67	23.61
3	4.07	klm	2–8	32.87	0.80	cdef	0.63–0.96	9.88	0.054	hijkl	0.02–0.11	35.32	2.66	abcd	1.42–3.88	23.67
4	5.13	defghijk	4–8	22.15	0.85	abcdef	0.65–1.83	30.08	0.063	defghijk	0.04–0.09	22.45	2.27	cdefgh	1.35–3.75	28.27
5	4.27	jklm	2–6	31.94	0.85	abcdef	0.74–1.03	8.91	0.055	hijkl	0.02–0.08	31.20	2.44	abcdefgh	1.31–5.43	36.21
6	4.07	klm	2–6	25.77	0.81	bcdef	0.57–1.00	12.70	0.050	jkl	0.02–0.08	28.89	2.41	bcdefgh	1.45–4.08	31.90
7	6.17	cdef	3–10	31.90	0.89	ab	0.76–1.09	9.76	0.070	cdefgh	0.03–0.11	29.29	2.36	cdefgh	1.68–3.29	20.16
8	4.07	klm	2–6	26.58	0.82	bcdef	0.72–0.98	8.43	0.051	ijkl	0.02–0.08	28.35	2.31	cdefgh	1.15–4.50	35.56
9	3.70	lm	2–6	26.70	0.83	abcdef	0.71–1.00	8.24	0.046	l	0.02–0.08	26.92	2.35	cdefgh	1.48–4.25	29.66
10	5.43	defghijk	2–8	27.20	0.81	bcdef	0.67–0.96	7.97	0.069	cdefgh	0.02–0.11	28.74	2.27	cdefgh	1.47–4.11	27.14
11	3.57	m	2–6	34.29	0.80	cdef	0.55–1.15	14.14	0.046	l	0.02–0.08	35.17	2.99	ab	1.63–6.00	40.49
12	4.47	hijklm	3–8	24.76	0.82	bcdef	0.67–0.98	8.85	0.061	efghijkl	0.04–0.10	24.59	1.96	ghi	1.00–2.86	22.68
13	5.67	cdefghi	2–8	30.86	0.90	ab	0.77–1.05	7.84	0.078	bcd	0.02–0.13	32.80	1.63	i	1.00–2.92	34.01
14	6.37	bcde	4–10	22.02	0.81	bcdef	0.63–1.05	14.14	0.074	bcdef	0.03–0.12	26.25	2.46	abcdefg	1.52–4.46	30.07
15	6.47	bcd	4–10	27.76	0.83	abcdef	0.68–1.07	9.99	0.078	bcd	0.04–0.13	28.43	1.85	hi	1.12–2.81	20.81
16	6.17	cdef	2–16	44.27	0.81	bcdef	0.59–0.96	10.63	0.073	cdef	0.02–0.17	41.84	2.20	cdefghi	1.55–3.50	22.26
17	5.33	defghijk	3–8	28.00	0.80	cdef	0.57–1.09	16.63	0.063	defghijk	0.04–0.10	26.90	2.98	ab	1.84–5.75	35.47
18	9.27	a	6–16	29.17	0.87	abcde	0.71–1.02	10.28	0.111	a	0.07–0.19	26.24	2.14	defghi	1.50–3.70	23.93
19	5.60	defghij	3–10	30.61	0.85	abcdef	0.76–1.00	8.12	0.072	cdefg	0.04–0.15	35.33	2.19	defghi	1.50–3.55	23.23
20	5.17	defghijk	3–8	24.95	0.83	abcdef	0.63–0.92	8.54	0.058	efghijkl	0.03–0.10	28.20	2.22	cdefghi	1.46–3.43	22.91
21	4.90	fghijklm	2–8	34.47	0.86	abcdef	0.53–1.16	16.31	0.054	hijkl	0.02–0.09	33.37	2.79	abc	1.94–5.38	29.16
22	5.30	defghijk	2–8	31.79	0.84	abcdef	0.53–1.14	14.20	0.069	cdefgh	0.02–0.11	32.94	2.65	abcde	1.39–5.75	40.51
23	5.07	efghijkl	2–10	33.96	0.82	bcdef	0.69–0.96	7.60	0.063	defghijk	0.03–0.11	32.84	2.04	fghi	1.50–3.46	26.99
24	5.97	cdefg	4–10	29.36	0.85	abcdef	0.75–1.00	7.61	0.075	bcde	0.04–0.13	29.53	2.10	defghi	1.50–3.50	20.85
25	4.93	fghijklm	2–10	29.62	0.82	bcdef	0.66–1.00	11.36	0.059	efghijkl	0.02–0.12	28.77	2.30	cdefgh	1.30–4.22	33.64
26	5.93	cdefg	3–10	36.74	0.87	abcde	0.66–1.13	11.73	0.065	cdefghijk	0.03–0.12	35.82	2.41	abcdefgh	1.55–5.63	37.27
27	4.87	fghijklm	2–10	33.14	0.82	bcdef	0.65–1.09	11.41	0.059	efghijkl	0.02–0.11	34.25	2.62	abcdef	1.64–4.78	37.97
28	5.33	defghijk	3–8	26.21	0.84	abcdef	0.71–0.92	6.50	0.066	cdefghij	0.03–0.12	30.13	2.45	abcdefg	1.71–4.10	26.00
29	5.20	defghijk	2–8	34.40	0.85	abcdef	0.73–0.98	7.19	0.065	cdefghijk	0.02–0.11	33.70	2.36	cdefgh	1.70–4.33	24.39
30	5.20	defghijk	4–8	27.85	0.80	cdef	0.58–1.00	12.43	0.058	efghijkl	0.04–0.11	29.05	2.67	abcd	1.73–4.75	27.17
31	7.60	b	4–18	34.13	0.82	bcdef	0.63–1.07	12.83	0.090	b	0.04–0.20	33.31	2.21	cdefghi	1.36–4.33	29.76
32	5.53	defghij	2–10	31.03	0.84	abcdef	0.65–1.09	12.25	0.064	cdefghijk	0.02–0.10	29.34	2.17	defghi	1.22–3.91	26.83
33	5.93	cdefg	2–10	31.28	0.82	bcdef	0.49–1.19	18.20	0.070	cdefgh	0.02–0.13	31.08	2.38	cdefgh	1.50–3.73	22.13

Table 3. *Cont.*

Genotype	Exp				P/E				Exp/P				A/B			
	Mean		**Range**	**cv**	**Mean**		**Range**	**cv**	**Mean**		**Range**	**cv**	**Mean**		**Range**	**cv**
34	4.43	hijklm	2–6	29.44	0.84	abcdef	0.65–1.38	15.72	0.052	ijkl	0.02–0.08	28.32	2.32	cdefgh	1.36–3.54	24.54
35	5.73	cdefgh	2–8	21.94	0.85	abcdef	0.72–1.00	9.71	0.069	cdefgh	0.02–0.10	22.91	2.42	abcdefgh	1.23–3.73	24.11
36	6.13	cdef	4–10	26.99	0.88	abc	0.70–1.07	11.69	0.072	cdefg	0.04–0.11	25.48	2.19	defghi	1.44–3.46	25.38
37	5.63	cdefghij	2–8	28.16	0.86	abcdef	0.07–1.00	9.55	0.068	cdefghi	0.02–0.01	26.66	2.06	efghi	1.5–3.545	26.35
38	4.63	ghijklm	2–6	24.37	0.81	bcdef	0.66–1.00	10.44	0.056	ghijkl	0.03–0.08	23.74	2.34	cdefgh	1.45–4.00	29.33
39	4.80	fghijklm	2–8	27.58	0.91	a	0.80–1.13	8.75	0.058	efghijkl	0.02–0.10	31.19	2.63	abcde	1.68–4.75	25.66
40	5.77	cdefgh	2–8	31.44	0.91	a	0.71–1.17	11.87	0.067	cdefghi	0.03–0.10	30.11	2.42	abcdefgh	1.30–5.00	32.13
41	5.40	defghijk	4–10	29.43	0.84	abcdef	0.56–1.05	11.52	0.069	cdefgh	0.04–0.13	27.78	2.21	cdefghi	1.47–3.46	27.48
42	5.50	defghij	2–8	24.69	0.84	abcdef	0.62–1.03	11.13	0.067	cdefghi	0.02–0.11	23.98	2.13	defghi	1.11–3.82	28.91
43	4.33	ijklm	2–6	29.29	0.79	ef	0.55–1.00	14.41	0.051	ijkl	0.02–0.08	30.54	2.22	cdefghi	1.15–4.50	32.14
44	4.07	klm	2–6	34.74	0.81	bcdef	0.44–1.00	15.38	0.046	l	0.02–0.09	39.97	3.00	a	1.67–5.63	31.00
45	4.97	fghijkl	3–8	30.14	0.86	abcdef	0.72–1.04	9.64	0.058	efghijkl	0.03–0.10	31.57	2.25	cdefgh	1.55–4.50	31.73
46	5.10	defghijk	2–8	25.41	0.84	abcdef	0.70–1.08	9.21	0.061	efghijkl	0.02–0.11	28.68	2.25	cdefgh	1.59–3.91	28.27
47	7.00	bc	4–10	24.60	0.87	abcd	0.59–1.07	15.28	0.081	bc	0.05–0.11	21.85	2.18	defghi	1.42–4.73	33.01
48	5.40	defghijk	2–10	39.09	0.84	abcdef	0.70–1.13	9.98	0.068	cdefghi	0.02–0.12	36.87	2.06	efghi	1.39–4.00	33.29
$LSD_{0.001}$	1.379				0.086				0.017				0.593			
ANOVA *F*	11.95 ***				2.83 ***				10.97 ***				4.63 ***			

LSD—least significant differences; *** $p < 0.001$.

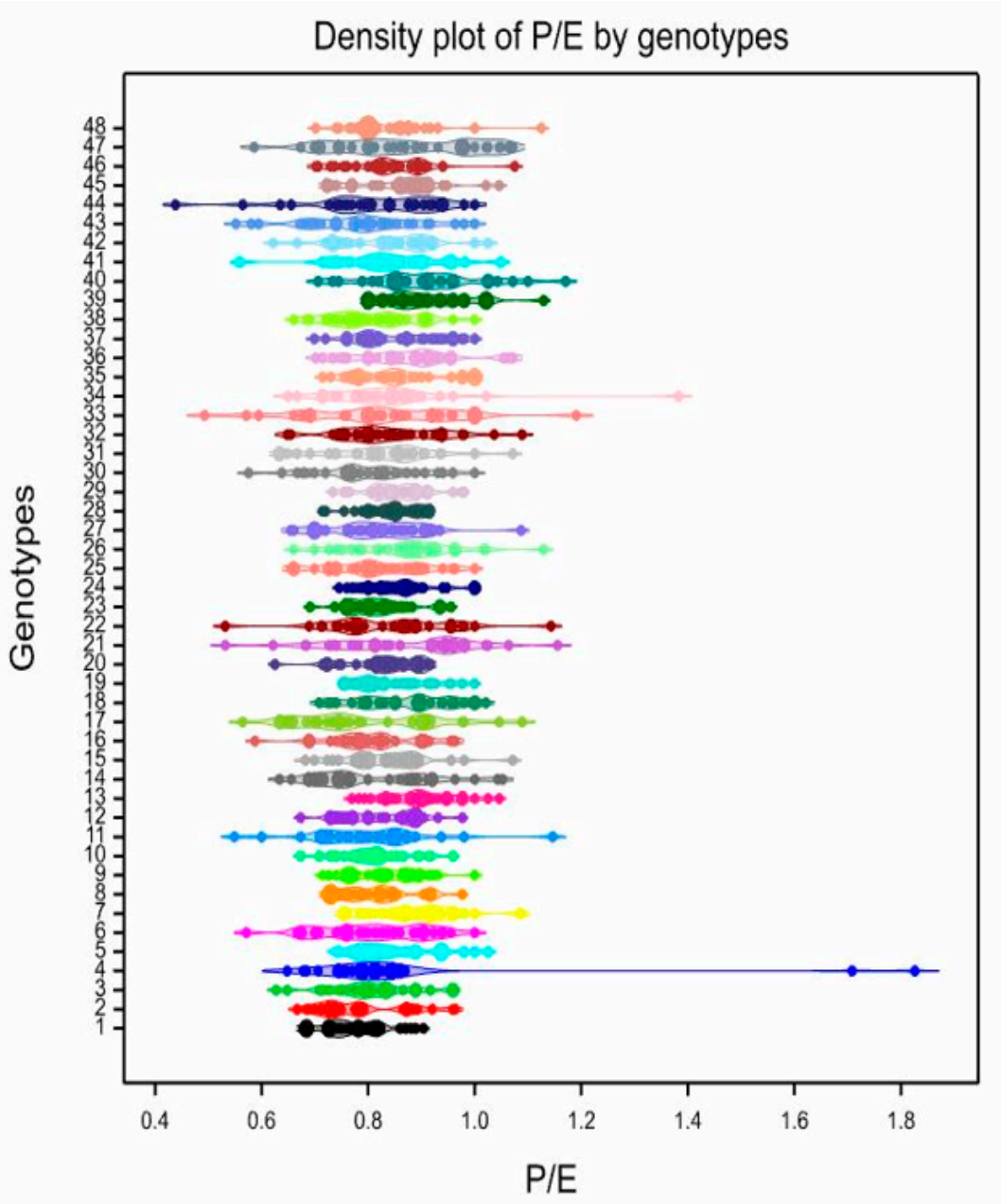

Figure 7. The density plot for the distribution of the shortest mean polar axis and the mean length of the equatorial diameter ratio (P/E), classified by *Abies alba* genotypes. Numbers from 1 to 48, see Table 1.

The exine surface was psilate, psilate with perforations and verrucate, or microverrucate. In the cappa area usually, a slightly more distinct exine surface (from psilate to verrucate or microverrucate) than in the leptoma area (psilate without perforations) was observed. The leptoma borders were verrucate or microverrucate. In the saccus, a mostly psilate exine surface, without or with perforations of different diameters were observed (Figure 4A–E).

The pollen grains of *A. alba* had two separate sacci, more or less equal in size. The average width of the saccus (A) was 73.46 µm, with extreme values of 40.00 µm in genotype 42 and 118.00 µm in genotype 36, and its average length (B) was 33.43 µm, (from 12.00 µm in genotype 11 to 62.00 µm in genotype 35). The mean A/B ratio (saccus shape) was 2.34 and ranged from 1.00 in genotypes 12 and 13 to 6.00 in genotype 11. This means that the saccus was usually much wider than being longer, elliptical, and flattened (Tables A2 and A3, Figure 4A–E and Figure 8).

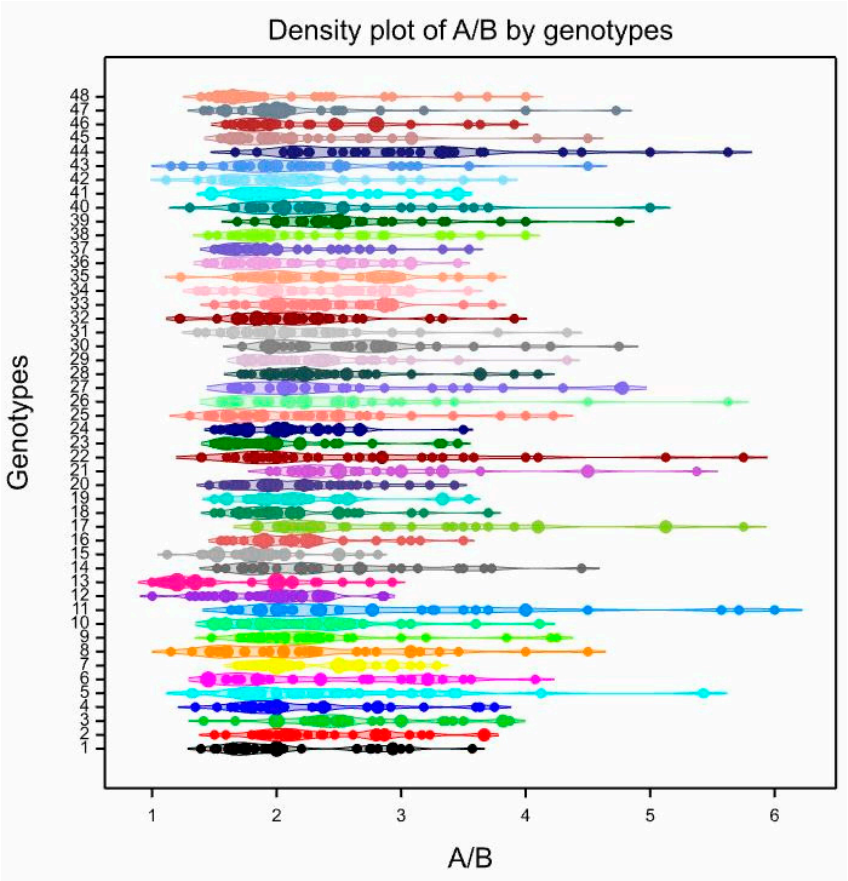

Figure 8. The density plot for the distribution of the average width of the saccus and average length ratio (A/B), classified by *Abies alba* genotypes. Numbers from 1 to 48, see Table 1.

The pollen grains under analysis usually had one aperture—leptoma. The leptoma was a thinning of the pollen wall on the distal face presumed to function as a germination area. It was located above the cappa (thick-walled proximal face of the corpus), and between the two sacci. The aperture was wide and trapezoidal in outline. The aperture membrane was usually thin, psilate without perforations, sometimes with single verrucae (>1 µm), or microverrucae (<1 µm), while the leptoma borders were usually covered with numerous verrucae or microverrucae.

3.3. Pollen Variability of the Studied Genotypes

The results of the MANOVA performed indicated that all *A. alba* genotypes were significantly different with regard to all eight quantitative traits (Wilk's l = 0.1998; $F_{47;1392} = 6.59$; $p < 0.0001$). The analysis of variance for the eight quantitative traits (P ($F_{47;1392} = 7.72$), E ($F_{47;1392} = 15.42$), A ($F_{47;1392} = 8.70$), B ($F_{47;1392} = 7.30$), Exp ($F_{47;1392} = 11.95$), P/E ($F_{47;1392} = 2.83$), Exp/P ($F_{47;1392} = 10.97$), and A/B ($F_{47;1392} = 4.63$) confirmed the variability of the tested genotypes at a significance level $\alpha = 0.001$ (Tables A2 and A3). The range, mean values, and coefficients of the variation for the observed traits indicated a high variability among the tested genotypes, for which significant differences were found in terms of all the analysed quantitative traits (Tables A2 and A3). The average P of the tested genotypes ranged from 73.07 (genotype 12) to 92.53 (genotype 26), with an average of 83.26 (Table A2, Figure 5). We obtained 12 homogeneous groups of the genotypes for this trait. The value of E ranged from 82.9 (genotype 13) to 113.9 (genotype 30), with an average of 100.3 (Table A2, Figure 6). We obtained 19 homogeneous groups of *A. alba* genotypes for E. The average A of the tested genotypes ranged from 64.4 (genotype 13) to 82.4 (genotype 29), with an average of 73.46 (Table A2). On the basis of obtained data, we observed 14 homogeneous groups of genotypes for trait A. The values of B ranged

from 26.27 (genotype 11) to 43.00 (genotype 13), with an average of 33.43 (Table A2). For this trait, we obtained 15 homogeneous groups of *Abies* genotypes.

The average Exp of the tested genotypes varied from 3.57 (genotype 11) to 9.27 (genotype 18), with an average of 5.3 (Table 3). We obtained 13 homogeneous groups of genotypes for Exp. The average P/E of the tested genotypes varied from 0.78 (genotype 1) to 0.91 (genotype 39), with an average of 0.84. Only six homogeneous groups of genotypes were obtained for P/E. The mean values of Exp/P ranged from 0.05 (genotypes 9, 11, and 44) to 0.11 (genotype 18), with an average of 0.06 (Table 3). We obtained 12 homogeneous groups of *A. alba* genotypes for Exp/P. The average A/B of the tested genotypes varied from 1.63 (genotype 13) to 3.00 (genotype 44), with an average of 2.34 (Table 3). The nine homogeneous groups of genotypes were obtained for trait A/B. The ranking of the variability of observed traits follows as Exp > Exp/P > A > P > B > A/B > P/E.

The correlation analysis performed indicated statistically significant correlation coefficients for 13 out of 28 coefficients (Figure 9). Trait P significantly correlated with E ($r = 0.800$), A ($r = 0.671$), and A/B (0.292). Additionally, E correlated with A (0.647), P/E (−0.531), and A/B (0.401). Exp significantly correlated with P/E (0.413), Exp/P (0.965), and A/B (−0.363). Trait A/B positively correlated with A (0.342) and negatively with B (−0.811) and Exp/P (−0.451). The positive correlation was observed between Exp/P and P/E (0.402) (Figure 9).

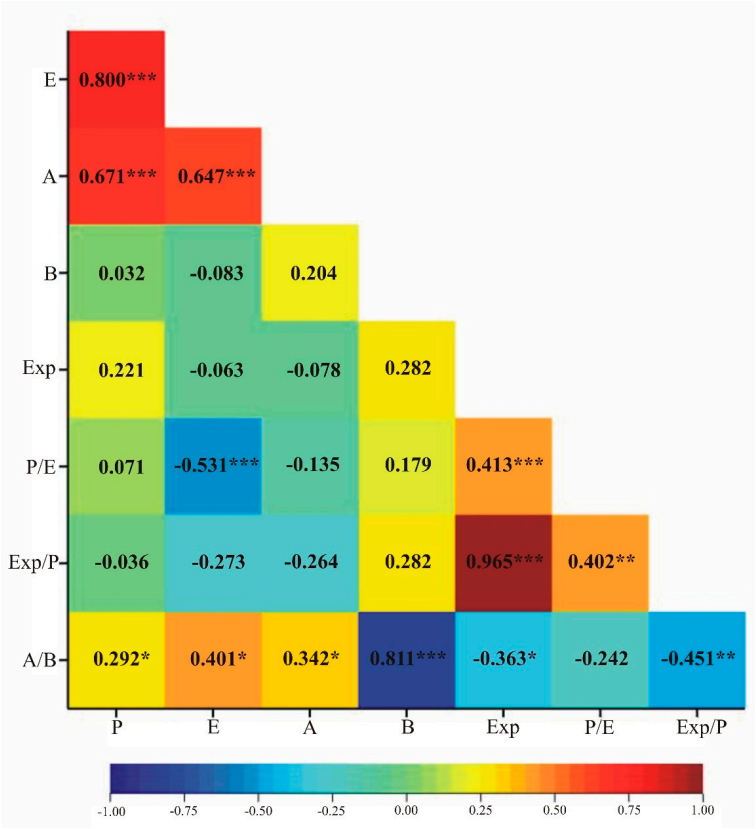

Figure 9. Heatmap for Pearson's linear correlation coefficients between observed traits (length of polar axis—P, equatorial diameter—E, width of the base of saccus—A, length of saccus—B, exine thickness—Exp, and P/E, Exp/P and A/B ratios) of *Abies alba* genotypes. A heatmap is a data visualisation technique that shows the magnitude of a phenomenon as colour in two dimensions. The variation in colour may be by hue or intensity, giving obvious visual cues to the reader on how the phenomenon is clustered or varies over space. Magnitudes are laid out into a matrix of fixed cell size, with rows and columns as observed traits, and the sorting of rows and columns is intentional and somewhat arbitrary, with the goal of suggesting clusters or portraying them as discovered via statistical analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the presented dendrogram, as a result of the nearest neighbour grouping using the Euclidean distances method, all examined *A. alba* genotypes were divided into four clusters (Figure 10). The first group contained only a single genotype, i.e., genotype 13, the second one genotypes 18 and 31, while the third cluster grouped four genotypes: 17, 21, 30, and 44. All remaining genotypes belonged to the large fourth cluster (Figure 10).

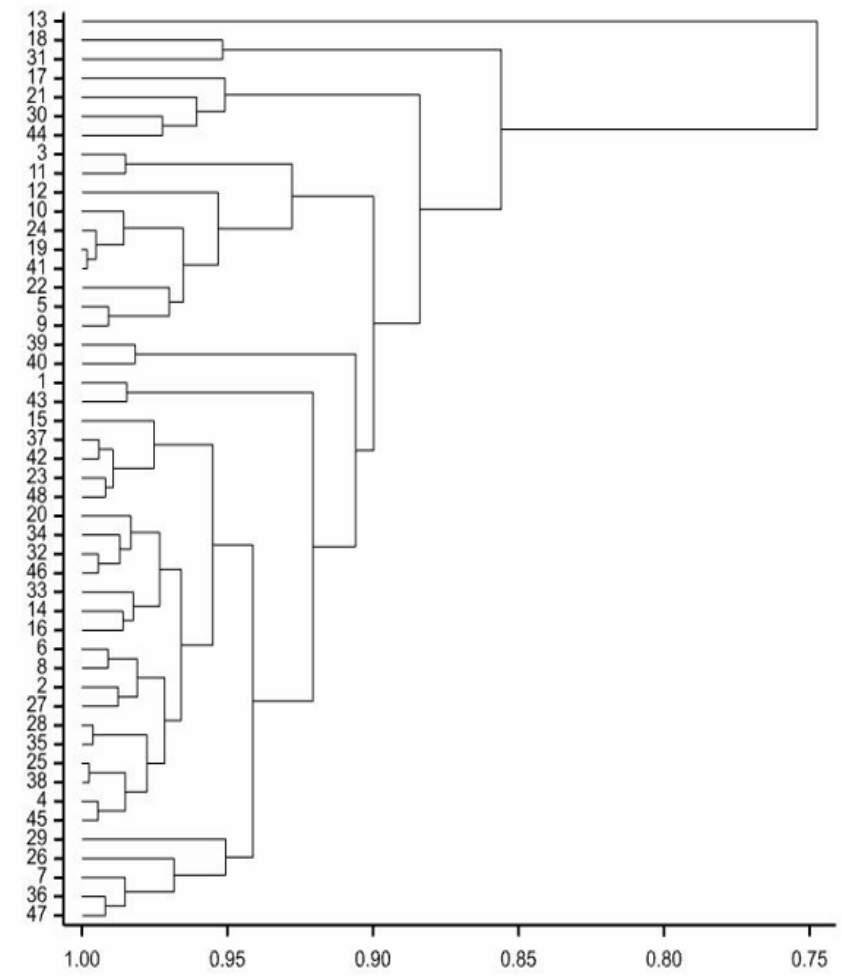

Figure 10. Dendrogram of differences between analysed *Abies alba* genotypes verified by the cluster analysis using the nearest neighbour method and Euclidean distances on the basis of eight traits (length of polar axis—P, equatorial diameter—E, width of the base of saccus—A, length of saccus—B, exine thickness—Exp, and P/E, Exp/P and A/B ratios). Numbers from 1 to 48, see Table 1.

Individual traits were of different importance and had a different share in the joint multivariate variation. The study on the multivariate variation for *A. alba* genotypes also included the identification of the most important traits in the multivariate variation of the genotypes. Figure 10 shows the variability of the eight quantitative traits of 48 studied *A. alba* genotypes. In the graph, the coordinates of the point for particular genotypes are the values for the first and second canonical variables. The first two canonical variables accounted jointly for 59.77% of the total multivariate variability between the individual genotypes (Table 4, Figure 11). The most significant, positive, linear relationship between the first canonical variable was found for P/E and Exp/P (Table 3). The first canonical variable negatively correlated with P, E, A and A/B (Table 4). The second canonical variable significantly positively correlated with P, B, Exp, P/E, and Exp/P and negatively correlated with A/B (Table 4). The greatest variation in terms of all eight traits (measured by Mahalanobis distances) was found for genotypes

13 and 30 (the Mahalanobis distance between them amounted to 4.69) (Table A3). The greatest similarity was found for genotypes 37 and 42 (0.44).

Table 4. Correlation coefficients between the first two canonical variables and original traits.

Trait	First Canonical Variable	Second Canonical Variable
P	−0.846 ***	0.355 *
E	−0.975 ***	0.035
A	−0.772 ***	0.045
B	0.051	0.533 ***
Exp	0.117	0.94***
P/E	0.417 **	0.441 **
Exp/P	0.343 *	0.873 ***
A/B	−0.436 **	−0.516 ***
Percentage variation	35.27	24.50

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

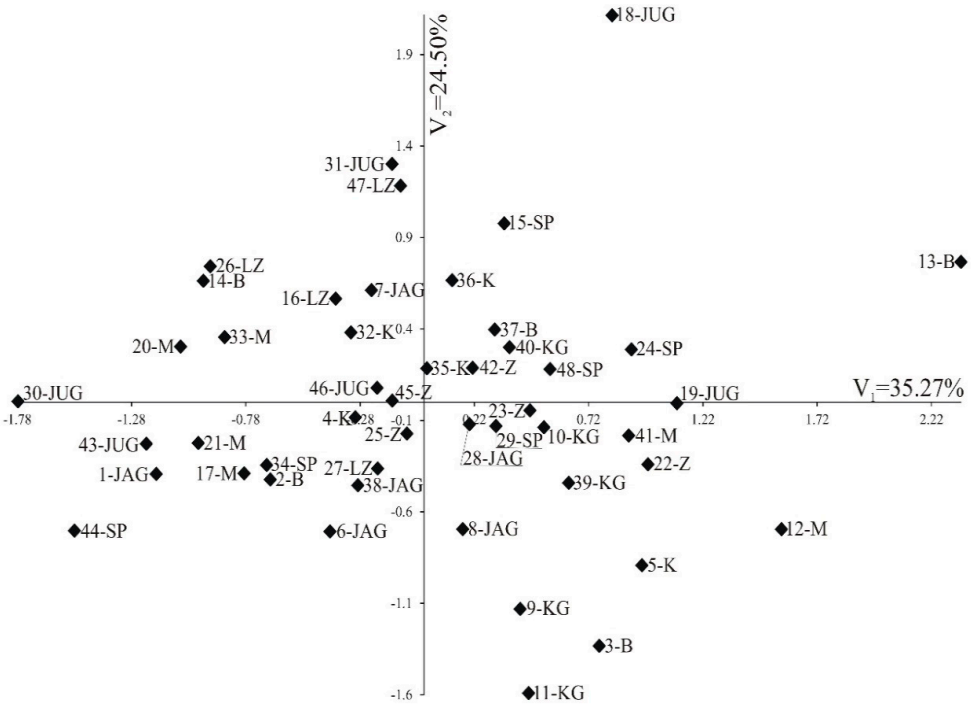

Figure 11. Distribution of 48 *Abies alba* genotypes in the space of the first two canonical variables. The coordinates of the point for particular genotypes are values of the first and second canonical variables, respectively. Numbers from 1 to 48, see Table 1.

4. Discussion

The results of our study indicate that the pollen grains of *Abies alba* are more or less similar to other species from the genus *Abies* originating from either Europe or Asia [18–20,22–24,44,45]. However, pollen grains of *A. alba* possess some distinct features, which will be further discussed. Firs have large or very large, saccate pollen grains with one aperture, the leptoma, and impression mark. Pollen grains are usually more or less flattened and have perforate psilate or verrucate exine sculpture.

The scarcity of investigations dealing with the characteristics of pollen in *A. alba* prevent a detailed analysis or comparisons. Some limited results are available, but mostly they focus on pollen size (P and E) and shape (P/E) [19,21]. Gudeski [19] examined and compared pollen grains of *A. alba* from populations in Macedonia and of *A. cephalonica* from Parnis in Greece. Dobrinov and Gagov [21] studied morphology, viability, and storage of pollen from four native populations of *A. alba* in Bulgaria.

However, the data provided by the authors on P, E, and the P/E ratio are consistent with the information presented in this paper.

Our studies allowed us to define a few diagnostic features of *A. alba* pollen grains. Most of these features are also mentioned by other palynologists, except for one (saccus shape—A/B ratio), which we have described for the first time. Those traits are the exine surface of pollen corpus (cappa and leptoma) and sacci, the length of the polar axis (P), pollen shape (P/E ratio), and the saccus shape (A/B ratio). The prominent feature for the species from the genus *Abies*, in comparison to other conifers (e.g., genera *Larix*, *Pinus*, and *Picea*), is the Y-shaped impression mark on the pollen proximal face and the separated sacci, but these traits are not species-specific and are therefore not suitable for species-level analysis [18,22–24,26]. Faegri and Iversen [26] underlined in their study that the exine on the pollen proximal view in *Abies* is of considerable thickness and attains more than 5 µm. Our results confirmed this and showed that on average the mean exine thickness in *A. alba* genotypes was 5.30 µm.

The *A. alba* genotypes analysed in this study were not significantly differentiated in terms of the exine surface. The available data on this trait concerns other *Abies* species, and thus cannot be used in direct comparisons. However, in most of these papers, two types of exine surface (psilate or verrucate) and its smaller or larger variation, depending on the pollen area (cappa, leptoma, and sacci), were reported [22–25]. Additionally, Khan et al. [25] in two Asiatic species (*A. pindrow* and *A. spectabilis*) recorded the rugulate exine ornamentation.

The length of the polar axis (P) allowed different clustering to be de defined among the investigated genotypes (large—50.01–100 µm and rarely very large >100.01 µm) and the range of this feature (Figure 4). A similar range of the polar axis length was noted by Halbritter [22–24] in *A. cephalonica*, *A. concolor*, and *A. nordmanniana*. Asiatic species *A. pindrow* and *A. spectabilis* produce much smaller pollen grains having the P range from 25 to 35 µm [25].

The studied genotypes differed also with respect to the pollen shape (P/E ratio). Most of the pollen grain was oblate with less frequently rounded pollen grains being noted and only sporadically were elongated ones found (genotype 4 or 34, Figure 5). The pollen grain shape has not been the objective of detailed investigations yet. In previous studies, we may find only results presenting the average value for the P/E ratio or the range of this trait among different *Abies* species without concluding on the exact shape [19,20,22–25]. For example, the measurement made on a few *Abies* species (*A. bracleafa*, *A. concolor*, *A. grandis*, *A. magnifica*, and *A. procera*) from the Californian Peninsula [18] indicated the predominance of the elongated pollen grains (average P/E ratio > 1.01). In most studies, including this one, oblate pollen grains were found to be the most frequent [20,22–25]. Based on this limited data we may conclude that the values of the P/E ratio reported here are comparable to published data, though in the case of our study the variability of this trait is much higher. This difference probably reflects the inter-species variability but may also stem from much wider sampling as each genotype was represented by 30 pollen grains, which was not a routine procedure in the past.

A new trait analysed in this study, the saccus shape (A/B), provided interesting results. Among the studied genotypes, none produced elongated sacci and only rarely could we find rounded ones (e.g., 12, 13, 42, and 43). On the other hand, in a majority of the investigated genotypes the sacci were flattened (1.5—three times wider than longer), and eight genotypes possessed significantly flattened sacci—even six times wider than longer (Figure 6). The variability of the A/B trait is also substantial and the greatest was noted in genotypes 11, 17, 22, and 44. This trait turned out to be useful because it enabled a few genotypes to be distinguished. Up to date, the results on the saccus width are available for *A. grandis* [18]. That work reported an average value of this feature to be 79.93 µm, which is close to our results obtained for *A. alba*—73.46 µm.

The results of the statistical analyses performed indicated that all studied *A. alba* genotypes were significantly different with regard to all eight quantitative pollen traits. Statistical analysis for these traits suggests a high variability among the tested genotypes. The most variable biometric traits were the equatorial diameter—E, exine thickness—Exp, and Exp/P ratio, while lower variability was found in the width of the saccus—A, length of the polar axis—P and length of the saccus—B, and the lowest

variability were noted for the A/B and P/E ratios (Table A2). The outstanding diagnostic value can be ascribed to trait E; the level of variability of this trait enabled to distinguish in *A. alba* genotypes a few pollen shape classes—from greatly oblate up to elongated.

Pollen grains from genotypes 13 (Bystrzyca Kłodzka) and 18 (from Jugów) were the most different because they had the smallest average values of traits P and E. In addition, four other genotypes (11—from Kamienna Góra, 30 and 31—from Jugów, and 44—from Szklarska Poręba) were slightly different from the others. Genotype 30 was distinguished by high average values of P and E, and 31 by P and P/E, while genotype 44 had significant values of P and E, and low values of feature A.

The analysis of localities from which the studied samples were collected showed (Figure 11) that similar pollen features occurred among samples collected from the same sites (e.g., 18 and 31 from Jugów) as well as from places distant from one another (e.g., 30 from Jugów and 44 from Szklarska Poręba). Similar results were obtained by Wrońska-Pilarek et al. [46,47] and Lechowicz et al. [48] in the palynological studies of selected species from the genera *Crataegus*, *Quercus*, and *Rubus*. In this way, results on the morphological variability correspond with genetic results, namely PCA performed on an individual level. However, the ordination of the seed orchards according to the first two axes in PCA revealed some differentiation between them, which was further supported by a significant genetic differentiation reaching 3%. However, it needs to be clearly stressed that the results of the genetic analysis based on 4–5 individuals should be treated with caution. On the other hand, even such a small sample was able to deliver some preliminary insights into the genetic relationships between the locations. The lack of full correspondence between morphological and genetic data is not surprising if we consider the nature of those two data sets. The principal cause is the polygenic character of the morphological traits and the impact of environmental conditions on trait expression. In contrast, the single-gene SSRs markers possess their own genealogy that is governed by different neutral evolutionary processes.

5. Conclusions

1. The most important pollen grain features of the studied *Abies alba* genotypes are comprised of exine surface of pollen corpus (cappa and leptoma) and sacci, the length of the polar axis (P), pollen shape (P/E ratio), and the new trait, saccus shape (A/B ratio).
2. The results of the presented study showed that the analysed morphological features of pollen grains from 48 A. alba genotypes did not provide grounds to distinguish individual genotypes, except for a few of them, but were generally their groups.
3. Nevertheless, in our opinion the pollen of *A. alba* was the source of important characteristics at the species and genotype level. These results of the first study on the pollen morphology and variability of *A. alba*, could support further research on the reproduction of this valuable forest tree species.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The analysed pollen grains for the studied genotypes of *Abies alba* individuals.

Number	Genotype Number	Object/Seed Orchard Location												
1	40,077	Karkonosze National Park Jagniątków	224	224	118	128	168	168	160	226	164 178	132	150
2	4064	Forest District Bystrzyca Kłodzka Pokrzywno	221	224	108	108	168	168	244	260	170 176	140	152
3	4144	Forest District Bystrzyca Kłodzka Pokrzywno	224	224	108	112	172	172	238	264	154 168	150	150
4	20,011	Karkonosze National Park Karpacz	224	224	110	116	170	172	160	236	166 194	146	146
5	10,041	Karkonosze National Park Karpacz	221	224	110	110	170	170	174	236	152 186	154	154
6	40,019	Karkonosze National Park Jagniątków	224	221	110	110	168	178	248	248	166 168	150	152
7	40,036	Karkonosze National Park Jagniątków	224	224	110	110	170	172	206	240	154 166	132	152
8	40,056	Karkonosze National Park Jagniątków	221	224	108	116	172	176	240	248	165 168	140	152
9	87	Forest District Kamienna Góra Ogorzelec	224	224	110	110	168	168	206	248	162 162	140	154
10	142	Forest District Kamienna Góra Ogorzelec	-	-	108	114	172	172	234	234	170 170	150	150
11	127	Forest District Kamienna Góra Ogorzelec	224	224	112	112	168	168	176	178	172 194	150	150
12	24	Forest District Śnieżka Maciejowa	-	-	110	112	168	170	206	248	172 186	150	150
13	4063	Forest District Bystrzyca Kłodzka Pokrzywno	224	224	110	110	168	168	188	206	154 166	140	148
14	4002	Forest District Bystrzyca Kłodzka Pokrzywno	224	224	110	110	168	178	236	240	152 170	148	148
15	60,046	Karkonosze National Park Szklarska Poręba	224	224	108	114	168	170	160	250	170 172	150	152

Table A1. *Cont.*

Number	Genotype Number	Object/Seed Orchard Location											
16	3021	Forest District Lądek Zdrój Trzebieszowice	206	221	110	110	176	176	174	246	152 170	140	140
17	198	Forest District Śnieżka Maciejowa	-	-	108	108	168	170	174	248	170 178	140	140
18	5341	Forest District Jugów Wojbórz	224	224	110	110	168	168	174	236	152 152	150	160
19	5335	Forest District Jugów Wojbórz	221	224	110	116	168	168	160	160	172 172	142	142
20	12	Forest District Śnieżka Maciejowa	-	-	110	110	168	170	160	206	170 194	138	148
21	235	Forest District Śnieżka Maciejowa	-	-	110	110	170	170	242	248	166 168	152	152
22	6031	Forest District Zdroje Duszniki	224	224	108	110	170	170	168	236	186 186	142	150
23	6078	Forest District Zdroje Duszniki	221	221	110	110	168	178	240	240	154 154	150	152
24	60,017	Karkonosze National Park Szklarska Poręba	221	224	108	108	168	168	242	248	166 176	152	152
25	6074	Forest District Zdroje Duszniki	224	224	110	112	168	176	250	250	166 166	142	142
26	3014	Forest District Lądek Zdrój Trzebieszowice	224	224	110	110	170	172	194	194	166 178	140	140
27	3158	Forest District Lądek Zdrój Trzebieszowice	221	221	112	112	168	176	240	248	152 170	140	140
28	40,073	Karkonosze National Park Jagniątków	224	224	110	116	168	170	174	242	168 168	140	152
29	60,135	Karkonosze National Park Szklarska Poręba	221	224	110	110	168	170	160	250	154 192	150	152
30	5347	Forest District Jugów Wojbórz	224	224	110	110	168	174	194	226	166 166	150	150
31	5262	Forest District Jugów Wojbórz	221	224	110	110	172	172	174	174	152 170	152	152
32	10,038	Karkonosze National Park Karpacz	221	224	108	108	168	170	194	250	152 194	140	144

Table A1. *Cont.*

Number	Genotype Number	Object/Seed Orchard Location												
33	78	Forest District Śnieżka Maciejowa	221	224	110	112	170	172	226	250	170 178	150	150
34	60,002	Karkonosze National Park Szklarska Poręba	221	224	-	-	172	172	178	242	178 178	148	154
35	30,006	Karkonosze National Park Karpacz	224	224	110	116	168	172	174	246	152 178	140	140
36	20,019	Karkonosze National Park Karpacz	224	224	110	110	168	168	242	250	152 178	140	146
37	4122	Forest District Bystrzyca Kłodzka Pokrzywno	224	224	110	110	174	174	206	226	166 196	140	152
38	40,049	Karkonosze National Park Jagniątków	224	224	110	110	172	172	242	250	152 166	132	152
39	65	Forest District Kamienna Góra Ogorzelec	224	224	110	110	172	172	236	236	186 186	146	152
40	15	Forest District Kamienna Góra Ogorzelec	221	224	110	110	168	168	236	250	166 166	146	146
41	133	Forest District Śnieżka Maciejowa	-	-	108	116	168	168	160	160	164 164	146	152
42	6056	Forest District Zdroje Duszniki	224	224	110	112	172	172	206	248	168 170	144	144
43	5254	Forest District Jugów Wojbórz	206	221	110	110	172	178	178	236	166 172	148	156
44	60,086	Karkonosze National Park Szklarska Poręba	224	224	110	110	170	170	240	250	178 178	152	152
45	6094	Forest District Zdroje Duszniki	224	224	114	114	172	172	248	248	176 176	150	150
46	5314	Forest District Jugów Wojbórz	224	224	108	116	168	170	206	206	152 172	140	140
47	3125	Forest District Lądek Zdrój Trzebieszowice	224	224	110	110	168	170	164	250	176 176	140	140
48	60,206	Karkonosze National Park Szklarska Poręba	224	224	110	110	168	170	160	160	180 180	140	150

"-"—failed amplification; probably null alleles

Table A2. Minimal, maximal, and mean values as well as coefficient of variation (cv, in %) for P, E, A and B. In columns, means followed by the same letters are not significantly different.

Genotype	P				E				A				B			
	Mean		Range	cv	Mean		Range	cv	Mean		Range	cv	Mean		Range	cv
1	84.33	bcdefghi	70–98	8.19	108.5	abcde	100–120	6.01	82.27	a	64–104	13.67	39.53	ab	28–50	15.13
2	83.53	bcdefghi	52–104	12.41	106.1	bcdefghi	70–116	7.90	76.07	abcdefghij	56–92	12.09	32.33	cdefghijklmn	24–44	16.73
3	75.47	jkl	56–90	10.85	94.7	opqr	70–108	8.04	71	fghijklmn	56–86	11.69	28	mno	16–48	23.73
4	82.6	efghi	68–104	10.77	101.1	fghijklmno	46–124	16.50	71.33	efghijklmn	60–90	12.37	33.13	cdefghijklm	20–52	22.79
5	78.33	hijkl	68–90	8.32	93	pqr	80–110	7.51	67.33	klmn	42–84	12.99	30	ijklmno	14–54	26.95
6	83	defghi	64–96	11.49	102.7	defghijklm	90–120	6.78	76.67	abcdefghi	58–114	20.25	33.47	cdefghijklm	22–44	19.15
7	88.4	abcde	76–102	7.71	99.6	ghijklmnopq	90–110	5.31	79.07	abcd	60–92	11.05	34.53	bcdefghijk	26–50	17.99
8	80	fghijkl	70–94	7.19	98.3	jklmnopq	84–106	5.82	76.07	abcdefghij	62–90	10.91	35.8	bcdefghi	20–54	24.70
9	80.27	fghijk	70–96	8.11	96.8	klmnopq	86–110	5.91	68.73	jklmn	52–100	15.80	30.47	ghijklmno	16–46	18.86
10	80	fghijkl	68–94	10.13	98.8	ijklmnopq	86–116	7.26	65.73	lmn	48–82	12.55	30.33	hijklmno	18–44	19.67
11	77.8	ijkl	56–98	11.08	97.8	klmnopq	82–112	6.50	70.27	hijklmn	58–80	9.80	26.27	o	12–38	29.23
12	73.07	l	62–84	8.28	89.1	rs	80–98	6.00	65.2	mn	52–80	12.31	34.27	bcdefghijkl	26–56	16.84
13	73.93	kl	64–90	7.67	82.9	s	70–96	7.19	64.4	n	54–86	11.22	43	a	24–60	27.90
14	87.8	abcde	76–120	10.99	108.9	abcd	94–126	8.08	76.73	abcdefghi	64–98	12.37	33.07	cdefghijklm	22–44	21.94
15	83.2	cdefghi	68–102	9.95	100.1	ghijklmnop	86–128	8.68	67.67	klmn	50–90	11.28	37.6	abcd	24–50	17.24
16	84.53	bcdefghi	66–104	10.74	104.1	cdefghijk	92–126	7.96	75.47	abcdefghij	64–90	10.69	35.33	bcdefghij	24–44	14.82
17	84.67	bcdefghi	70–100	8.69	107.9	abcdef	86–128	11.33	73.53	cdefghijk	62–92	10.42	26.67	no	16–38	24.15
18	83.4	bcdefghi	68–100	9.57	96.2	lmnopqr	90–106	4.71	69.4	ijklmn	56–84	9.99	33.47	cdefghijklm	20–40	15.21
19	78.8	ghijkl	68–100	9.53	93.1	pqr	82–108	6.93	65.07	mn	50–80	13.40	30.73	fghijklmno	18–52	20.90
20	89.87	abcd	76–108	7.97	108.8	abcd	96–128	6.43	76	abcdefghij	60–96	11.63	35.4	bcdefghij	26–48	16.60
21	90.13	abc	56–108	11.98	106.2	bcdefgh	60–132	14.20	77.47	abcdefgh	64–90	7.70	29.4	klmno	16–36	20.62
22	78.13	hijkl	50–128	16.63	93.2	pqr	80–112	6.96	71.8	defghijklmn	56–92	13.21	29.87	jklmno	16–46	25.96
23	80.13	fghijk	64–92	7.27	97.6	klmnopq	84–110	6.82	70.47	ghijklmn	60–86	8.15	36.27	bcdefg	20–48	19.68
24	80.4	fghijk	66–96	8.27	94.7	opqr	78–110	8.54	65.2	mn	54–84	11.49	31.93	defghijklmno	24–44	18.50
25	83.53	bcdefghi	70–106	10.83	102.3	defghijklmn	90–116	5.53	70.2	hijklmn	60–94	11.70	32.93	cdefghijklm	18–50	25.46
26	92.53	a	72–124	12.87	106.9	abcdefg	92–124	7.20	78.93	abcde	62–96	12.41	35.13	bcdefghijk	16–46	20.37
27	83.67	bcdefghi	68–100	11.44	102.3	defghijklmn	90–116	6.18	74.87	abcdefghijk	60–92	12.70	31.07	efghijklmno	16–44	24.07
28	82.13	efghij	68–96	7.73	98	klmnopq	84–114	7.47	75.8	abcdefghij	60–90	9.58	32.33	cdefghijklmn	20–42	19.84
29	80.6	fghijk	70–98	7.26	94.6	opqr	80–112	6.92	82.4	a	64–104	10.99	36.27	bcdefg	24–48	18.24
30	90.33	ab	74–110	9.47	113.9	a	100–132	8.16	80.93	abc	64–96	8.87	31.87	defghijklmno	16–44	19.36
31	84.87	bcdefgh	70–104	9.11	104.1	cdefghijk	84–120	8.54	70.8	fghijklmn	60–92	12.40	33.67	bcdefghijklm	18–44	19.80
32	86.4	abcdef	66–114	11.60	102.7	defghijklm	90–116	7.00	74.73	abcdefghijk	56–92	12.22	35.87	bcdefgh	22–52	18.34
33	85.13	bcdefgh	70–116	11.91	105.5	bcdefghij	84–142	12.23	81.6	ab	64–112	15.22	35.27	bcdefghij	24–48	18.33

Table A2. *Cont.*

Genotype	P				E				A				B			
	Mean		**Range**	**cv**	**Mean**		**Range**	**cv**	**Mean**		**Range**	**cv**	**Mean**		**Range**	**cv**
34	85.73	abcdefg	74–102	8.39	103.5	cdefghijkl	68–120	9.08	78.93	abcde	60–100	14.44	35.13	bcdefghijk	26–46	14.70
35	84.07	bcdefghi	74–100	6.17	99.1	hijklmnopq	80–112	8.49	76.2	abcdefghij	56–90	11.52	33	cdefghijklm	20–62	23.75
36	85.07	bcdefgh	66–116	10.88	97.5	klmnopq	84–112	7.66	78.13	abcdefg	66–118	15.28	37.07	bcd	22–48	17.68
37	83.47	bcdefghi	70–98	8.56	97.8	klmnopq	84–114	6.16	72.33	defghijklm	60–88	10.53	36.4	bcdef	22–46	15.57
38	83.47	bcdefghi	70–104	9.60	103.2	cdefghijklm	80–114	8.01	71.53	defghijklmn	54–102	16.17	31.8	defghijklmno	20–42	16.79
39	84.27	bcdefghi	58–102	11.04	92.6	qr	62–110	9.99	72.13	defghijklmn	58–88	10.24	28.53	lmno	16–40	18.77
40	85.8	abcdefg	70–100	7.70	95.1	nopqr	80–116	9.95	73.4	cdefghijkl	54–90	12.44	32.47	cdefghijklmn	14–46	24.51
41	78.4	hijkl	64–108	10.87	94.6	opqr	76–136	12.93	65.27	mn	50–90	15.53	30.8	fghijklmno	18–42	20.58
42	82.6	efghi	66–94	8.51	99.3	hijklmnopq	80–112	6.31	70.87	fghijklmn	40–84	13.84	34.8	bcdefghijk	20–46	18.21
43	86	abcdef	62–102	12.61	110.3	abc	96–132	8.97	77.2	abcdefgh	60–102	13.82	36.87	bcde	20–52	21.45
44	89.93	abcd	64–110	12.37	112.1	ab	100–146	9.81	78.33	abcdef	60–96	11.81	27.87	mno	16–48	24.93
45	86.27	abcdef	74–100	7.20	101.3	efghijklmno	80–130	10.50	71	fghijklmn	54–90	12.44	33.4	cdefghijklm	20–48	22.69
46	84.47	bcdefghi	72–100	7.48	100.9	fghijklmno	80–114	7.61	77.27	abcdefgh	66–92	8.90	36.2	bcdefg	22–46	19.65
47	86.33	abcdef	70–110	10.07	100.3	ghijklmnop	82–140	12.37	76.33	abcdefghij	64–104	11.29	37.33	abcd	22–54	22.87
48	79.73	fghijkl	66–90	8.09	95.9	mnopqr	80–110	8.58	73.87	bcdefghijk	64–96	11.68	37.87	abc	22–48	18.34
$LSD_{0.001}$	7.053				7.337				7.739				5.863			
ANOVA *F*	7.72 ***				15.42 ***				8.70 ***				7.30 ***			

LSD—least significant differences; *** $p < 0.001$.

Table A3. Mahalanobis distances between studied *Abies* genotypes (G).

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2	1.63																						
3	2.73	1.91																					
4	2.35	1.65	2.35																				
5	2.77	1.97	0.94	2.01																			
6	1.33	0.72	1.64	1.71	1.65																		
7	1.97	1.68	2.40	1.98	2.13	1.58																	
8	1.48	1.53	1.54	2.05	1.48	0.91	1.69																
9	2.43	1.66	1.08	1.99	0.76	1.30	2.06	1.40															
10	2.86	1.53	1.67	1.82	1.32	1.73	1.98	2.03	1.44														
11	2.90	2.26	1.19	2.42	1.29	1.99	2.79	1.86	1.49	2.08													
12	2.99	2.35	1.34	2.48	1.03	2.02	2.40	1.66	1.41	1.68	2.10												
13	4.11	3.89	3.68	3.53	3.15	3.65	3.56	3.14	3.66	3.36	3.87	2.71											
14	2.11	1.30	2.73	1.69	2.56	1.75	1.50	2.25	2.47	1.84	2.85	3.01	3.99										
15	2.62	1.94	2.64	1.91	2.10	2.05	1.56	2.12	2.25	1.45	2.88	2.22	2.80	1.56									
16	1.82	1.13	2.29	1.68	2.10	1.36	0.95	1.68	2.04	1.54	2.65	2.36	3.51	0.84	1.16								
17	2.67	1.73	2.31	1.66	2.24	2.00	2.37	2.42	2.24	1.90	1.92	3.03	4.31	1.53	2.36	1.91							
18	4.00	3.22	3.53	3.06	3.20	3.46	2.40	3.49	3.53	2.48	3.89	3.25	3.65	2.46	1.92	2.27	3.16						
19	3.21	1.98	1.83	2.09	1.41	2.07	2.16	2.21	1.71	0.76	2.41	1.43	2.94	2.32	1.60	1.90	2.49	2.45					
20	1.72	1.14	2.78	1.82	2.50	1.40	1.50	2.02	2.19	1.95	2.93	2.91	3.98	1.04	1.66	1.10	2.05	3.09	2.39				
21	2.02	1.16	2.36	1.38	2.14	1.34	1.63	1.98	1.90	1.84	2.30	2.85	4.12	1.17	2.04	1.43	1.27	3.19	2.32	1.11			
22	2.82	1.85	1.35	2.15	1.25	1.77	2.07	1.64	1.73	1.32	1.82	1.40	2.75	2.29	1.91	1.86	2.24	2.72	1.19	2.50	2.23		
23	1.97	1.46	1.66	1.73	1.23	1.19	1.35	0.99	1.32	1.31	2.06	1.35	2.78	1.85	1.21	1.18	2.23	2.69	1.48	1.71	1.84	1.35	
24	3.07	1.92	1.97	1.90	1.44	2.02	1.87	2.16	1.71	0.68	2.46	1.57	2.98	2.04	1.22	1.62	2.34	2.13	0.50	2.15	2.13	1.38	1.30
25	2.04	1.09	1.78	1.47	1.35	1.10	1.49	1.43	1.27	1.00	1.88	1.91	3.34	1.37	1.26	1.09	1.57	2.76	1.52	1.25	1.19	1.55	0.88
26	1.91	1.64	2.94	2.11	2.60	1.75	1.13	2.09	2.45	2.19	3.02	3.04	3.86	1.07	1.57	1.09	2.16	2.74	2.56	0.85	1.34	2.51	1.77
27	1.88	0.81	1.58	1.57	1.45	0.85	1.49	1.24	1.40	1.25	1.72	2.01	3.43	1.34	1.64	1.11	1.45	2.91	1.68	1.39	1.10	1.29	1.08
28	1.98	1.08	1.49	1.74	1.42	0.99	1.18	1.16	1.45	1.29	2.08	1.64	3.20	1.61	1.59	1.02	2.04	2.64	1.43	1.67	1.58	1.09	0.89
29	1.87	2.00	2.07	2.52	2.19	1.61	1.58	1.23	2.22	2.53	2.74	2.05	3.26	2.50	2.44	1.84	3.03	3.28	2.49	2.46	2.50	1.90	1.60
30	1.80	1.31	2.96	1.95	2.93	1.70	2.02	2.41	2.62	2.45	2.95	3.49	4.69	1.07	2.44	1.57	1.64	3.50	2.99	1.13	1.06	2.86	2.37
31	2.95	2.07	2.92	2.02	2.59	2.43	1.74	2.69	2.74	1.70	3.12	2.89	3.63	1.14	1.14	1.22	2.00	1.41	2.02	1.87	2.00	2.24	1.94
32	1.70	1.18	2.27	1.63	1.91	1.18	0.86	1.45	1.79	1.56	2.53	2.23	3.32	1.13	1.10	0.61	2.02	2.57	1.88	0.89	1.29	1.88	0.95
33	1.44	1.30	2.50	1.67	2.50	1.41	1.21	1.74	2.36	2.24	2.82	2.79	3.91	1.17	1.99	0.95	2.02	2.86	2.56	1.45	1.49	2.28	1.72
34	1.08	0.98	2.11	1.61	2.01	0.63	1.29	1.15	1.66	2.04	2.42	2.36	3.76	1.61	2.03	1.25	2.14	3.40	2.35	1.19	1.26	2.14	1.35
35	1.92	1.17	1.79	1.56	1.59	1.13	0.80	1.30	1.61	1.38	2.23	1.90	3.26	1.32	1.37	0.72	1.91	2.40	1.59	1.46	1.38	1.37	0.90
36	1.91	1.77	2.36	2.00	2.08	1.59	0.66	1.46	2.14	2.01	2.80	2.15	3.00	1.70	1.41	1.02	2.57	2.39	2.06	1.72	1.94	1.84	1.12
37	2.04	1.51	2.07	1.71	1.60	1.37	0.98	1.34	1.67	1.41	2.46	1.73	2.81	1.60	0.92	0.93	2.30	2.38	1.51	1.48	1.73	1.53	0.56
38	1.92	0.85	1.62	1.44	1.39	0.88	1.52	1.43	1.06	1.13	1.86	1.97	3.73	1.45	1.66	1.18	1.57	3.02	1.68	1.26	1.07	1.73	1.11
39	2.75	1.74	1.54	1.75	1.07	1.56	1.72	1.72	1.23	1.28	1.98	1.57	3.19	2.22	1.93	1.84	2.23	2.93	1.18	2.13	1.73	1.24	1.37
40	2.42	1.79	2.00	1.52	1.45	1.63	1.02	1.62	1.63	1.49	2.26	1.88	3.01	1.74	1.33	1.30	2.08	2.34	1.57	1.80	1.56	1.54	1.06

Table A3. *Cont.*

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
41	2.95	1.79	1.52	1.72	1.03	1.82	1.98	1.94	1.34	0.58	2.01	1.30	3.06	2.13	1.53	1.72	2.12	2.49	0.58	2.24	2.04	1.17	1.22	
42	2.06	1.29	1.83	1.50	1.38	1.23	1.11	1.34	1.41	1.08	2.18	1.68	3.04	1.44	0.95	0.84	1.96	2.40	1.36	1.40	1.51	1.44	0.52	
43	1.12	1.09	2.66	1.73	2.53	1.16	1.92	1.72	2.21	2.27	2.69	2.93	3.96	1.42	2.10	1.45	1.98	3.63	2.72	0.95	1.30	2.55	1.79	
44	2.40	1.49	2.87	2.06	2.83	1.85	2.67	2.58	2.56	2.40	2.66	3.49	4.62	1.76	2.78	2.21	1.58	3.99	2.90	1.66	1.21	2.72	2.61	
45	2.10	1.22	2.08	1.38	1.55	1.21	1.35	1.59	1.41	1.20	2.23	2.06	3.32	1.40	1.22	1.14	1.83	2.78	1.57	1.07	1.11	1.77	1.02	
46	1.40	1.10	2.05	1.70	1.83	0.86	1.00	1.00	1.73	1.75	2.41	2.04	3.16	1.47	1.48	0.88	2.20	2.88	1.96	1.23	1.48	1.68	0.87	
47	2.29	2.13	2.83	1.92	2.45	2.12	1.03	2.06	2.58	2.20	3.01	2.68	3.20	1.47	1.26	1.13	2.34	1.98	2.38	1.82	1.97	2.25	1.56	
48	1.92	1.75	1.92	1.95	1.63	1.43	1.34	0.98	1.81	1.81	2.35	1.55	2.53	2.02	1.43	1.31	2.53	2.69	1.83	1.99	2.14	1.43	0.64	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	

	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
25	1.31																							
26	2.25	1.46																						
27	1.60	0.67	1.54																					
28	1.38	1.08	1.72	0.79																				
29	2.46	2.18	2.36	1.88	1.30																			
30	2.79	1.81	1.46	1.66	2.08	2.79																		
31	1.65	1.69	1.69	1.86	1.89	2.81	2.19																	
32	1.60	0.85	0.87	1.03	1.05	1.83	1.67	1.55																
33	2.35	1.69	1.45	1.47	1.37	1.67	1.38	1.88	1.22															
34	2.21	1.31	1.47	1.18	1.20	1.58	1.53	2.37	1.02	1.08														
35	1.40	1.00	1.39	0.86	0.47	1.42	1.89	1.58	0.76	1.11	1.11													
36	1.82	1.56	1.42	1.52	1.08	1.23	2.30	1.83	0.94	1.28	1.37	0.81												
37	1.24	0.97	1.41	1.18	0.90	1.63	2.25	1.65	0.64	1.55	1.34	0.72	0.76											
38	1.51	0.54	1.61	0.76	1.08	2.14	1.65	1.94	1.02	1.59	1.10	1.05	1.70	1.23										
39	1.22	1.35	2.22	1.32	1.13	2.05	2.62	2.33	1.63	2.21	1.76	1.25	1.76	1.38	1.35									
40	1.28	1.16	1.60	1.30	1.10	1.86	2.35	1.72	1.07	1.71	1.55	0.83	1.07	0.82	1.34	1.04								
41	0.55	1.21	2.41	1.43	1.28	2.37	2.75	1.91	1.69	2.31	2.09	1.38	1.94	1.36	1.32	1.05	1.31							
42	1.08	0.62	1.45	0.94	0.83	1.81	2.06	1.52	0.65	1.52	1.29	0.66	1.06	0.44	0.88	1.27	0.82	1.10						
43	2.56	1.44	1.46	1.40	1.80	2.36	1.11	2.36	1.30	1.39	1.02	1.69	1.95	1.78	1.39	2.38	2.12	2.48	1.67					
44	2.85	1.91	2.12	1.68	2.30	3.20	1.24	2.70	2.15	2.21	1.97	2.28	2.85	2.59	1.82	2.50	2.61	2.72	2.37	1.50				
45	1.32	0.52	1.30	0.96	1.19	2.22	1.82	1.74	0.73	1.70	1.24	1.03	1.47	0.89	0.74	1.25	1.01	1.34	0.69	1.46	1.95			
46	1.80	1.09	1.27	0.97	0.80	1.31	1.82	1.97	0.63	1.12	0.75	0.71	0.81	0.75	1.17	1.58	1.21	1.79	0.86	1.28	2.21	1.06		
47	2.01	1.71	1.35	1.82	1.67	2.05	2.25	1.34	1.19	1.42	1.85	1.25	0.95	1.20	1.95	2.20	1.24	2.16	1.33	2.11	2.92	1.65	1.42	
48	1.69	1.39	1.89	1.38	1.01	1.20	2.57	2.10	1.18	1.64	1.48	1.00	0.84	0.75	1.61	1.66	1.21	1.65	0.96	1.97	2.89	1.48	0.87	1.42
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47

$D_{0.05} = 2.73$.

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