



Variability in the quality of pollen grains in oat amphiploids and their parental species

Paulina Tomaszewska^{1,2} · Romuald Kosina³

Received: 19 October 2021 / Revised: 13 July 2022 / Accepted: 14 July 2022 / Published online: 2 August 2022
© The Author(s) 2022

Abstract

The pollen analysis has become an important technique to evaluate the use of selectively breeding crops, including the three most important grasses to human nutrition. This study aimed to evaluate the reproductive potential of oat species and their hybrid progeny (amphiploids) during three consecutive vegetation seasons. Correlation and regression analyses were used to describe the morphotypes and viability of pollen grains, while numerical taxonomy methods were applied to analyse the relationships between taxa. The results indicated a difference in the size of pollen grains between the growing seasons, but a stable association between the taxa. The viability of pollen grains showed no correlation with pollen length. In the ordination space, amphiploids and parental species were well discriminated. Amphiploids and parental species were characterised by a positive correlation between the pollen size and the level of ploidy; however, along the respective regression line, the amphiploids were located among species with a high level of ploidy. Developmental anomalies of pollen grains were more frequent in amphiploids, with few pollen grains being chromosomally imbalanced, and the formation of micrograins was the most common event. Multiporate pollens being of multiple-spindle mother cells origin can be equivalents of four monoporate microspores. A strong correlation was observed between the frequencies of multiporate grains and micropollens. In the ordination space, monoporate types (species) were discriminated from multiporate types (amphiploids). High viability of pollen in amphiploids proved their genomic/chromosomal stabilisation across many generations of reproduction.

Keywords *Avena* · Correlation · Developmental anomalies · Multiporate pollen · Numerical taxonomy · Regression

1 Introduction

For understanding the mating system in plants, especially their common hybrid swarms, research on the development and variability of pollen grains is important. Due to differences in the mating systems, the variability and differentiation processes can vary in wild or cultivated plant populations (Grant 1981; Richards 1986). Natural and/or artificial selections throughout generations in cereals, the most important grasses to mankind, stabilise the reproduction process at

the even number of chromosomes (Kushwaha et al. 2004). Wild and cultivated oats, as well as their artificial hybrids, were also affected by these changes.

The pool of viable pollen grains available during the flowering time is crucial to reproductive success. Regular meiosis, which is typical for specific taxa, ensures successful reproduction; however, this process can be irregular in species, but especially in hybrids. In meiosis of *Avena* L. species (Sheidai et al. 2003) and cultivars of *Avena sativa* L. (Baptista-Giacomelli et al. 2000a, b) or *Urochloa decumbens* (Stapf) R.D. Webster (previously classified as *Brachiaria decumbens* Stapf; Mendes-Bonato et al. 2002), various anomalies related to chromosomes or activity of karyokinetic spindle were observed. These anomalies often resulted in the formation of cytoplasmic separations with micronuclei and, ultimately, micropollens. In interspecific and intergeneric hybrids, meiotic disturbances were more severe and frequent (Mujeeb et al. 1978; Linde-Laursen and von Bothmer 1993; Li et al. 2005); however, they showed a similar pattern. Thus, studies that focus on the frequency of

✉ Romuald Kosina
romuald.kosina@uwr.edu.pl

¹ Department of Genetics and Cell Physiology, Faculty of Biological Sciences, University of Wrocław, 50-328, Wrocław, Poland

² Department of Genetics and Genome Biology, University of Leicester, Road, Leicester LE1 7RH, UK

³ Faculty of Biological Sciences, University of Wrocław, Przybyszewskiego 63, 51-148, Wrocław, Poland

micronuclei and micropollen formation can be a useful tool for assessing the reproductive quality of a plant. Even in one anther sac, where the microsporocyte mother tissue forms a mosaic of cells with different numbers of chromosomes, meiotic disorders can be found. Such an event was noted in the amphiploid *Avena abyssinica* × *Avena strigosa* (Thomas and Peregrine 1964) and in *Allium senescens* subsp. *montanum* (Pohl) Holub (synonym of accepted *Allium senescens* L.) (Małecka 2008).

Studies have shown a change from generative to apomictic pattern of reproduction in species with high incidence of polyploidy or hybrids, including amphiploids with a double chromosome set (Grant 1981; Quarin et al. 2001; Ma et al. 2009). Unique morphogenesis of pollen grains in the form of multiporate units has been associated with apomixis (Ma et al. 2009). Due to the germination of pollen tubes through several pores and their mutual competition during tube growth, multiporate grains were considered to be ineffective in the process of reproduction (Florek 2013; Kosina et al. 2014). The metabolism and deposition of callose that occur before porus morphogenesis probably determine the pattern of porus formation (Teng et al. 2005). Studies have also shown that callose deposition in pollen grains differed among grasses (Warzych 2001; Kłyk 2005).

It is critical to assess the reproductive stability of plant hybrids and their relatives for breeding purposes. Therefore, this study aimed to compare the quality of pollen grains in oat species, showing a broad spectrum of ploidy and their amphiploids. The mating system of oat species and amphiploids was assessed considering the morphology and viability of pollen grains as indicators of hybrid vigour.

2 Materials and methods

Oat amphiploids and their parental species (Table 1) were cultivated on small plots in R. Kosina's grass collection (Wrocław, SW Poland). The plants were grown under the same soil–climatic conditions throughout the plot experiments. For three consecutive growing seasons, mature stamens were collected from plants at the turn of June and July. Each year, mature stamens were collected from five plants from each of the 15 accessions (taxa). Each taxon was treated as an Operational Taxonomic Unit (OTU) in numerical taxonomic analyses. Stamens were always collected from the lower flower of spikelet. Pollen grains isolated from anther were placed on microscope slides, stained with 0.5% (v/v) acetocarmine or 0.01% (v/v) acridine orange, mounted in glycerin and covered with a coverslip.

The viability of the pollen grains was determined using the acetocarmine staining technique, based on which the grains were classified into three groups:

- Normal, viable pollen: stained red, completely filled with cytoplasm
- Dysfunctional, defective pollen: weakly coloured, partially filled with cytoplasm
- Empty, dead pollen: uncoloured, with no cytoplasm, usually wrinkled or cracked.

Defective and dead pollen grains were classified as non-viable pollens.

Table 1 List of studied accessions of oat amphiploids and their parental species

Amphiploids and parental species	Abbreviation	Accession number	Origin	Ploidy level
<i>A. barbata</i> × <i>A. sativa</i> subsp. <i>nuda</i>	<i>b/sn</i>	CIav7903**	–	octoploid
<i>A. eriantha</i> × <i>A. sativa</i>	<i>e/sa</i>	PI458781**	–	octoploid
<i>A. barbata</i> × <i>A. sativa</i>	<i>b/sa</i>	CIav7901**	–	hexaploid
<i>A. fatua</i> × <i>A. sterilis</i>	<i>f/ste</i>	CIav9367**	–	hexaploid
<i>A. magna</i> × <i>A. longiglumis</i>	<i>m/l</i>	CIav9364**	–	hexaploid
<i>A. abyssinica</i> × <i>A. strigosa</i>	<i>a/str</i>	CIav7423**	–	tetraploid
<i>A. fatua</i>	<i>Af</i>	RK	Poland	hexaploid
<i>A. sterilis</i>	<i>Aste</i>	PI311689**	Israel	hexaploid
<i>A. sativa</i>	<i>Asa</i>	RK	Poland	hexaploid
<i>A. barbata</i>	<i>Ab</i>	AVE1938*	Spain	tetraploid
<i>A. abyssinica</i>	<i>Aa</i>	PI331373**	Ethiopia	tetraploid
<i>A. magna</i>	<i>Am</i>	1786***	Morocco	tetraploid
<i>A. strigosa</i>	<i>Astr</i>	51,624*	Belgium	diploid
<i>A. longiglumis</i>	<i>Al</i>	PI367389**	Portugal	diploid
<i>A. eriantha</i>	<i>Ae</i>	CIav9051**	England	diploid

*Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Braunschweig, Germany; **National Small Grains Collection, Aberdeen, Idaho, USA; ***Vavilov Institute of Plant Industry, St. Petersburg, Russia; RK–collected by R. Kosina

Table 2 Viability and size of pollen grains of oat amphiploids and their parental species

Amphiploid and parental species	Viability of pollen grains [%]				Length of pollen grains [µm]			
	Year 1	Year 2	Year 3	Mean	Year 1	Year 2	Year 3	Mean
<i>A. barbata</i> × <i>A. sativa</i> subsp. <i>nuda</i>	–	90.5	91.2	91.1	–	48.8	48.1	48.3
<i>A. eriantha</i> × <i>A. sativa</i>	84.4	98.3	97.6	90.5	53.9	50.5	56.0	53.9
<i>A. barbata</i> × <i>A. sativa</i>	92.8	96.8	96.7	96.0	42.0	41.5	46.8	43.6
<i>A. fatua</i> × <i>A. sterilis</i>	82.7	98.0	98.0	95.5	43.3	49.5	50.4	48.3
<i>A. magna</i> × <i>A. longiglumis</i>	96.6	95.6	91.1	95.1	51.8	46.6	50.5	49.8
<i>A. abyssinica</i> × <i>A. strigosa</i>	96.5	88.3	98.6	94.2	44.3	36.9	43.7	43.4
<i>A. fatua</i>	98.4	98.7	99.4	98.9	45.4	44.9	49.1	46.7
<i>A. sterilis</i>	96.9	99.5	98.8	98.5	50.8	50.1	50.2	50.4
<i>A. sativa</i>	99.0	88.5	98.2	96.5	43.0	46.5	40.9	43.0
<i>A. barbata</i>	97.6	–	99.0	98.1	44.5	–	43.7	44.0
<i>A. abyssinica</i>	96.3	–	97.7	97.4	45.3	–	43.5	44.0
<i>A. magna</i>	98.1	97.7	98.6	98.2	45.2	41.9	49.5	45.8
<i>A. strigosa</i>	96.6	99.1	98.9	98.3	44.1	42.7	45.2	44.0
<i>A. longiglumis</i>	–	98.1	95.5	96.3	–	42.9	44.5	44.3
<i>A. eriantha</i>	98.1	–	97.5	97.6	41.0	–	36.9	37.6

The species investigated were classified according to the nomenclature adopted from.

<https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch.aspx> (accessed on 21 July 2021) and <http://www.theplantlist.org/> (accessed on 21 July 2021):

- A. fatua* L.
- A. sterilis* L.
- A. sativa* L.
- A. barbata* Pott ex Link.
- A. abyssinica* Hochst.
- A. magna* H.C. Murphy & Terrell.
- A. strigosa* Schreb.
- A. longiglumis* Durieu.
- A. eriantha* Durieu.

Microscopy – The slides were examined under an Olympus BX-60 epifluorescence microscope (Hamburg, Germany), using both white and ultraviolet lights. An Olympus E-520 camera was used to capture images (Olympus Imaging Europe, Hamburg, Germany).

Statistical analysis – OTUs (15 accessions of oats) were described based on the following characteristics of pollen grains:

- Viability: all viable, dysfunctional and dead pollen grains taken from anther were counted
- Length of the pollen grain: measured for 40 randomly selected pollen grains from anther
- Impaired pollen grains: all micrograins, unreduced pollen grains and grains of irregular shape were counted
- Number of poruses in pollen grains.

Non-metric multidimensional scaling (nmMDS) (Kruskal 1964; Rohlf 1981) and Rohlf’s numerical approach (Rohlf 1994) were used to arrange the OTUs into the ordination space. KWIKSTAT 4 procedure (Elliot 1994) was used to conduct the correlation and regression analyses.

3 Results

Viability and size of pollen grains – The results of analyses with regard to the percentage of viable pollen grains in the analysed accessions throughout the course of three growing seasons are given in Table 2. The results revealed a high level of pollen grain viability for accessions of both amphiploids and their parental species. In all examined accessions, the average percentage of viable pollen for three years was above 90%. Amphiploids showed slightly lower viability of pollen grains compared with their parental species. The percentage of viable pollen in parental species ranged from 96.3 to 98.9%, and in amphiploids from 90.5 to 96.0%. Octoploid amphiploids showed the lowest pollen viability: the average percentage of pollen completely filled with cytoplasm in *b/sn* and *e/sa* was 91.1 and 90.5%, respectively. In certain growing seasons, some species and amphiploids, for example: *e/sa* and *f/ste* in year 1, and *a/str* and *Asa* in year 2, showed pollen grain viability below 90% or pollen was not collected for analysis due to seed germination problems or premature drying of plants caused by drought. Between the three growing seasons, the amphiploid *f/ste* (from 82.7 to 98.0%) and hexaploid species *Asa* (from 88.5 to 99.0%) showed the greatest range of pollen viability variation.

The pollen grains of amphiploids and their parental species vary in size. The pollen grains of oats are usually slightly elongated and elliptical in shape. Hence, their length was used as a measurement to describe the size of pollen grains, and these data are given in Table 2. The average length of grains ranged from 37.6 to 53.9 μm . The smallest grains were observed in *Ae*, while the largest pollen was found in the octoploid *e/sa*. In all the growing seasons, the mean length of the elliptical grains in the amphiploid *e/sa* was found to be above 50 μm . The size of pollen grains varied not only between the analysed accessions but also between years.

Pearson's correlation coefficients matrix of the pollen grain characteristics (pollen viability and their average length in particular growing seasons) is presented in Table 3. The results showed a correlation between the average lengths of pollen grains in three consecutive growing seasons. This significant correlation in the size of pollen grains between different growing seasons showed that this feature remained a stable characteristic of the taxa analysed in this study. A lack of correlation between the viabilities of pollen grains in individual years suggested a random, non-directional, high environmental variation of this feature. The matrix of Pearson's correlation coefficients of pollen grain characteristics was used to distribute the 15 studied oat accessions (OTUs) in the ordination space along three axes (x, y, z) using the Kruskal's nmMDS. The diagram of the minimum spanning tree (MST) (Fig. 1) shows the location of OTUs connected to each other by the smallest links. As shown in the diagram, the smallest distance was observed between the OTUs *Aa* and *Ab*. The inter-variety variability of the cultivated species *Asa* was the probable factor that located it in the vicinity of the taxon *Ae* with small pollen grains. The extreme position of the amphiploid *e/sa* was mainly determined by its largest pollen grains. The large distance between the two amphiploids *e/sa* and *f/ste*, which showed similar viability of pollen grains in different growing seasons, was due to the difference in the size of pollen grains in both taxa. There exists a certain directional difference between the species and their progeny—the species formed a cluster in the centre, while the amphiploids were scattered outside (Fig. 1). This means

that the pattern of seasonal variation of two analysed characteristics varied between oat species and amphiploids; however, there was not complete discrimination as the species *Aa* and *Ae* were located outside of the centre (Table 4

Regression of pollen length versus ploidy – The research showed a significant correlation between the length of pollen grains and the level of ploidy of the root tissue (Table 1; P. Tomaszewska, pers. comm.). For a set of OTUs ($n = 15$), Pearson's correlation coefficient (r) was 0.78, which shows a significant regression relationship between the variables (Fig. 2). Lower values of both characteristics were observed in di- and tetraploid species, while hexaploid species and amphiploids showing higher values were located in the right part of the diagram. In addition, for the extreme OTUs such as *e/sa* and *b/sn*, a high frequency of multiporate pollen grains was noted (see Tables 5 and 6).

Anomalous pollen grains – A part of grains, apart from normal pollen grains, showed developmental disorders, and the examples are shown in Fig. 3. All the accessions examined contained pollen micrograins (Fig. 3a); however, their percentage share of the total pollen grains analysed did not exceed 1% (Table 4). *Astr* (diploid species), as well as *e/sa*, *m/l* and *a/str* (amphiploids) showed very large, unreduced pollen grains (Fig. 3b, d, e, f). Their considerably large size could indicate a higher level of ploidy in pollen stem cell or non-reduction of gametes. This might be due to disturbances in the formation of cell walls during subsequent cytokineses that takes place during the process of microsporogenesis, which was confirmed as observed by the irregular shape and depressions on the surface of large pollen grains (Fig. 3c, g, h). However, the frequency of formation of large pollen grains was low and did not exceed 1%. The irregular shape of pollen grains may suggest an asymmetric division that led to the formation of micropollens (Fig. 3j). In the amphiploid *e/sa*, a single occurrence of a huge, highly elongated pollen was recorded (Fig. 3k), which was estimated to be about four to five times larger than a normally developed grain and had seven poruses, including two that were connected. Not only all amphiploids but also one hexaploid and three tetraploid

Table 3 Pearson's correlation coefficients matrix of the two pollen grain characteristics, viability and length, in the three growing seasons ($n = 15$, pooled sample of species and amphiploids)

Traits	V1	V2	V3	L1	L2	L3
V1	1.00					
V2	−0.24	1.00				
V3	−0.01	0.25	1.00			
L1	−0.26	0.31	−0.32	1.00		
L2	−0.46	0.27	−0.28	0.60*	1.00	
L3	−0.58*	0.57*	−0.11	0.73***	0.55*	1.00

V1, V2, V3—viability of pollen grains in the three growing seasons; L1, L2, L3—length of pollen grains in the three growing seasons; *, ***—significance levels at $\alpha = 0.05, 0.001$, respectively

Fig. 1 Minimum spanning tree (MST) of amphiploids and parental species (Operational Taxonomic Units, OTUs) of the genus *Avena* in the ordination space (x, y and z axes) created by using Kruskal's nmMDS method (Rohlf 1994). The OTUs were described by pollen viability and pollen length in the three growing seasons. Abbreviations are given in Table 1. The distances between pairs of OTUs as shown by the MST are as follows (extreme distances are in bold font): *b/sn*–*m/l* 0.869; *m/l*–*Aste* 0.775; *Aste*–*Am* 0.703; *Am*–*Af* 0.245; *Af*–*Astr* 0.398; *Astr*–*Aa* 0.469; ***Aa*–*Ab* 0.205**; *Astr*–*Al* 0.489; *Al*–*b/sa* 0.296; *Aa*–*a/str* 0.602; *a/str*–*Ae* 0.664; *Ae*–*Asa* 0.743; *Al*–*f/ste* 1.040; ***f/ste*–*e/sa* 1.231**

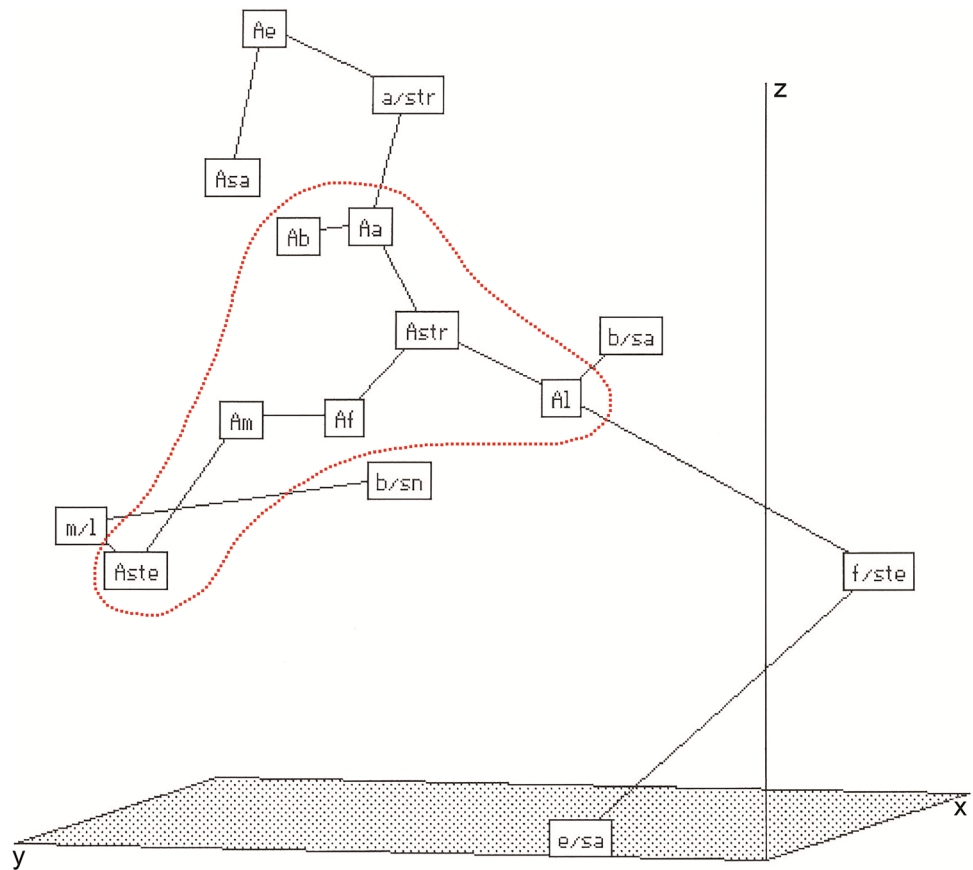


Table 4 Frequency of anomalous pollen grains (three-year average)

Amphiploids and parental species	Anomalous pollen grains [%]	
	Micropollens	unreduced irregular
<i>A. barbata</i> × <i>A. sativa</i> subsp. <i>nuda</i>	0.89	0 0.05
<i>A. eriantha</i> × <i>A. sativa</i>	0.62	0.02 0.06
<i>A. barbata</i> × <i>A. sativa</i>	0.28	0 0.01
<i>A. fatua</i> × <i>A. sterilis</i>	0.90	0 0.01
<i>A. magna</i> × <i>A. longiglumis</i>	0.12	0.01 0.02
<i>A. abyssinica</i> × <i>A. strigosa</i>	0.22	0.02 0.01
<i>A. fatua</i>	0.18	0 0.01
<i>A. sterilis</i>	0.30	0 0
<i>A. sativa</i>	0.19	0 0
<i>A. barbata</i>	0.03	0 0.02
<i>A. abyssinica</i>	0.46	0 0.03
<i>A. magna</i>	0.17	0 0.002
<i>A. strigosa</i>	0.24	0.01 0
<i>A. longiglumis</i>	0.09	0 0
<i>A. eriantha</i>	0.23	0 0

species showed pollen grain anomalies (Table 4). However, these anomalies accounted for only a small percentage of the total pollen pool analysed. The highest amount

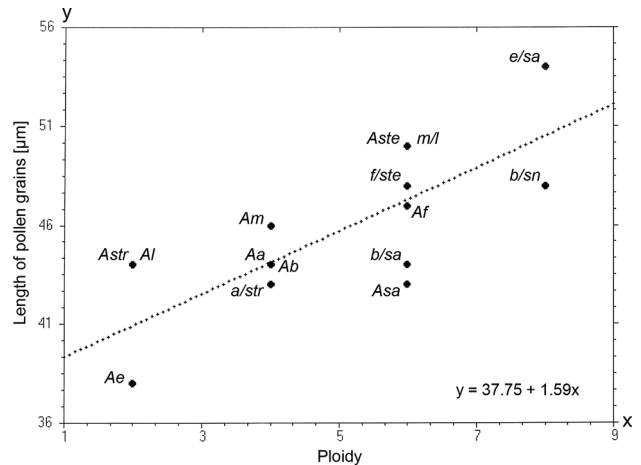


Fig. 2 Diagram of the linear regression showing the relationship between the length of pollen grain and level of ploidy level in oat amphiploids and parental species. Length of pollen grains expressed in micrometre (µm). R² (coefficient of determination)=0.61 indicates the good fit the regression line to the data

of irregularly shaped pollen grains was observed in octoploids, but their frequency in *e/sa* and *b/sn* was only 0.06 and 0.05%, respectively. The most common anomaly observed was the occurrence of micrograins. The average frequency

Table 5 Frequency of pollen grains with a certain number of poruses (%)

Amphiploids and parental species	1 porus	2 poruses	3 poruses	4 poruses	> 4 poruses
<i>A. barbata</i> × <i>A. sativa</i> subsp. <i>nuda</i>	97.1	2.28	0.54	0.13	0
<i>A. eriantha</i> × <i>A. sativa</i>	99.4	0.43	0.11	0	0.05
<i>A. barbata</i> × <i>A. sativa</i>	99.9	0.11	0	0	0
<i>A. fatua</i> × <i>A. sterilis</i>	99.4	0.62	0	0	0
<i>A. magna</i> × <i>A. longiglumis</i>	99.8	0.22	0	0	0
<i>A. abyssinica</i> × <i>A. strigosa</i>	99.8	0.21	0	0	0
<i>A. fatua</i>	100	0	0	0	0
<i>A. sterilis</i>	100	0	0	0	0
<i>A. sativa</i>	100	0	0	0	0
<i>A. barbata</i>	100	0	0	0	0
<i>A. abyssinica</i>	100	0	0	0	0
<i>A. magna</i>	100	0	0	0	0
<i>A. strigosa</i>	100	0	0	0	0
<i>A. longiglumis</i>	100	0	0	0	0
<i>A. eriantha</i>	100	0	0	0	0

Table 6 Pearson's correlation coefficient matrix for the ten characteristics of pollen grains (three growing seasons)

Traits	V	L	MP	UR	IR	1P	2P	3P	4P	>4P
V	1.00									
L	-0.51*	1.00								
MP	-0.66**	0.42	1.00							
UR	-0.52*	0.33	0.03	1.00						
IR	-0.78***	0.58*	0.59*	0.37	1.00					
1P	0.73***	-0.31	0.73***	0.01	-0.64**	1.00				
2P	-0.73***	0.32	0.75***	-0.01	0.62**	-1.00***	1.00			
3P	-0.67**	0.28	0.63**	-0.03	0.65**	-0.98***	0.96***	1.00		
4P	-0.56*	0.16	0.57*	-0.15	0.52*	-0.96***	0.95***	0.98***	1.00	
>4P	-0.56*	0.59*	0.30	0.60*	0.67**	-0.10	0.08	0.13	-0.07	1.00

V—viability of pollen grains, L—length of pollen grains, MP—micropollens, UR—unreduced pollen grains, IR—irregular pollen grains, 1P—pollens with one porus, 2P—pollens with two poruses, 3P—pollens with three poruses, 4P—pollens with four poruses, >4P—pollens with more than four poruses

of occurrence of micrograins in amphiploids was 0.51%, while a distinctly lower frequency of 0.21% was observed in the parental species.

Number of poruses in cell walls of pollen grains – As shown in Fig. 3, irregularly shaped pollen grains often had more than one porus. The number of poruses was investigated under UV light after staining the pollens with acridine orange. The results showed that normal pollen grains of the oat accessions analysed had contained one porus (Fig. 4a). However, all the amphiploids tested, including their normal pollen grains, showed the presence of two sporadic poruses, but this was absent in the parental species. The poruses differed from each other with regard to position—they were found to be distant (Fig. 4c) or close from each other (Fig. 4e) and also connected to each other (Fig. 4b, d). Further, the diameters of both poruses were similar or

different (Fig. 4c, f). A single occurrence of pollen grains with three poruses (Fig. 4f) and four poruses was reported in the amphiploid *b/sn*. Pollen grains with a higher number of poruses were also reported in amphiploid *e/sa*. An extreme case of multiporate grain having seven pores was observed in this amphiploid (Fig. 3k). Pollen with three and four poruses were not observed in the remaining four amphiploids. The frequency of pollen grains with an increased number of poruses was low (Table 5). *b/sn* showed the highest frequency of pollen grains with many poruses in comparison with other amphiploids.

Correlations between the characteristics of pollen grains – Pearson's correlation coefficients matrix was calculated for all the pollen characteristics (arithmetic averages from two or three consecutive growing seasons). The viability of pollen grains was positively correlated only with pollen grains

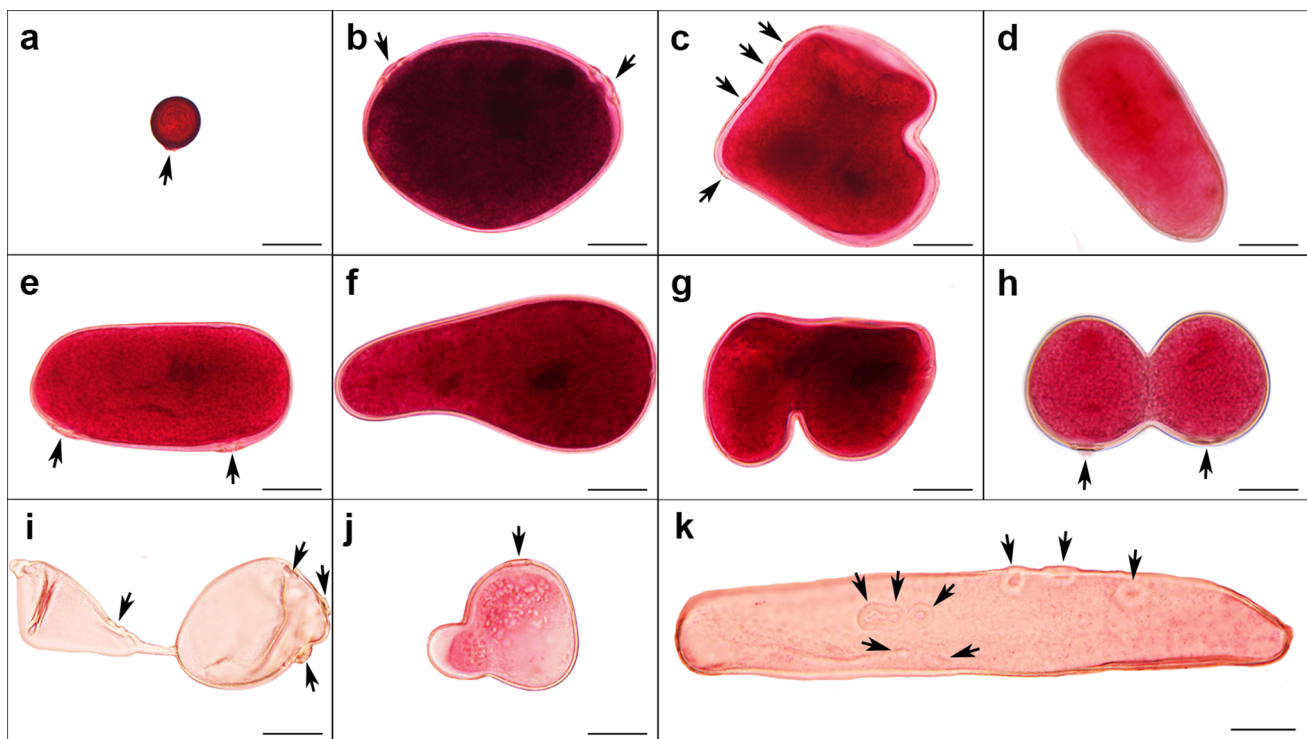


Fig. 3 Anomalous morphotypes of pollen grains. a – *A. fatua* × *A. sterilis*; b, c – *A. eriantha* × *A. sativa*; d – *A. abyssinica* × *A. strigosa*; e – *A. eriantha* × *A. sativa*; f, g – *A. eriantha* × *A. sativa*; h – *A. abyssinica* × *A. strigosa*; i – *A. barbata* × *A. sativa*; j – *A. fatua* × *A. sterilis*; k – *A. eriantha* × *A. sativa*. Acetocarmine staining, black arrows indicate poruses. Scale bars = 20 μm

having one porus (Table 6). Negative correlation coefficients between pollen viability and all other characteristics were also observed. This means that taxa producing micropollens, non-reduced pollen grains, irregular pollens and multiporate grains will have a lower ability to germinate pollen grains. This shows that disturbances in the development of pollen significantly affect their viability. The viability of the pollen was also negatively correlated with the length of the pollen grains. In comparison with diploid species producing haploid pollen grains, plants with a higher level of ploidy and consequently larger pollen grains showed reduced pollen viability. The presence of pollen micrograins was found to be positively correlated with irregular, three- and four-porus pollen and negatively correlated with one-porus pollen. Further, there was a positive correlation between the presence of pollen with more than four poruses and pollen grain length and non-reduced pollen. Other characteristics such as irregular pollen and the length of the pollen grains, as well as multiporate pollen grains, were also positively correlated. However, the presence of irregular pollen was found to be negatively correlated with one-porus grain. Comparison between the characteristics of pollen grains and the number of poruses showed a negative correlation between one-porus pollen and two-, three- and four-porus pollens. Frequency of two-, three- and four-porus grains was positively correlated.

These findings clearly indicate that the larger size of pollens and the higher level of ploidy, the greater the disturbance in the preceding microsporogenesis process leading to the development of anomalous pollen grains.

– The above can prove that large, multiporate, irregular pollen grains are units equivalent to four monoporate microspores developing abnormally in the form of an unreduced single cell, which shows a disturbed pattern of pores.

– Pearson's correlation coefficient matrix calculated for 10 pollen grain characteristics (Table 6) was used to distribute the 15 studied oat accessions (OTUs) in the space of three ordination axes using the Kruskal's nmMDS. The MST (Fig. 5) shows the location of the OTUs. The nmMDS analysis revealed significant variability between the taxa studied. In the MST diagram, diploid *Ae* and two octoploids *b/sn* and *e/sa* occupy opposite locations. The very small size of pollen grains, as well as the complete absence of non-reduced and irregular pollen grains in the pollen pool analysed from three growing seasons, determined the location of *Ae* in the diagram. The other two diploids, *Al* and *Astr*, also showed no significant disturbances in the development of pollen grains; however, the size of the pollen grains influenced the placement of the OTUs as shown in the diagram. *Astr* was located close to the tetraploid species *Am* and *Aa*. Further, *Al* showed values similar to traits of the amphiploid

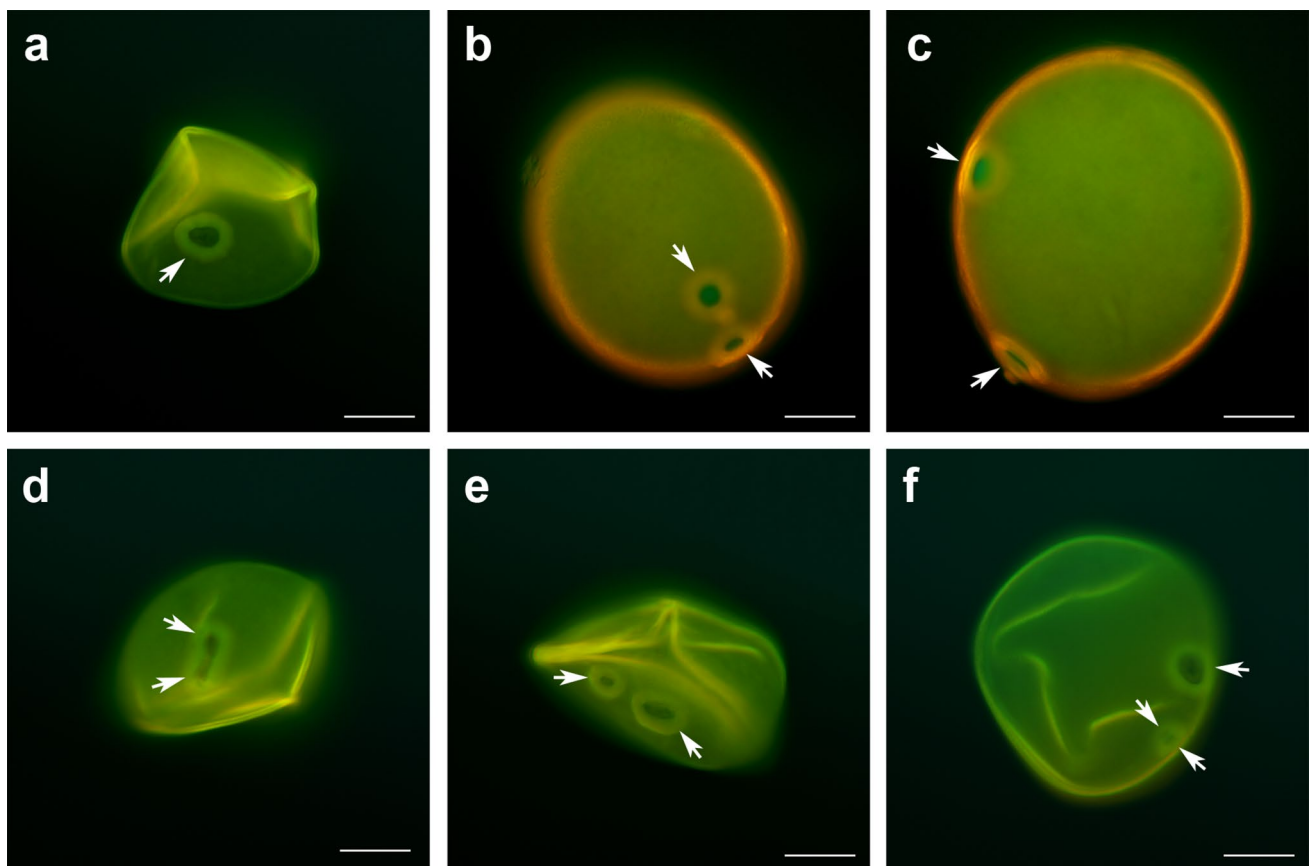


Fig. 4 Different types of morphotypes and number and position of poruses in pollen of oat amphiploids. a—*A. magna* × *A. longiglumis*, pollen with one porus; b, c—*A. eriantha* × *A. sativa*, pollens with two poruses; d—*A. magna* × *A. longiglumis*, pollen with two poruses; e—*A. magna* × *A. longiglumis*, pollen with two poruses; f—*A. barbata* × *A. sativa* subsp. *nuda*, pollen with three poruses. Acridine orange fluorescence. White arrows indicate poruses. Scale bars = 20 μm

b/sa. The phenotypic distance between these two OTUs was very small. The octoploids formed an extreme cluster in the diagram. In comparison with the other accessions, the location of octoploids in the ordination space was determined by their slightly reduced viability of pollen grains and higher frequency of irregular pollen grains. Moreover, both extreme amphiploids (*b/sn*, *e/sa*) were distinguished from other taxa by the higher frequency of multiporate pollens. Despite this, there was clear discrimination between both OTUs. It may result from significant differences in the size of pollen grains between the accessions of the species and amphiploids. The length of the pollen grains was found to be greatest in the amphiploid *e/sa*. Further, *b/sn*, despite having the same level of ploidy as *e/sa*, produced pollen that was comparable in size to that of hexaploid amphiploids. Differences in the percentage share of micropollens and non-reduced pollen grains in the pools analysed could possibly influence the distribution of both OTUs. The smallest distance between *Ab* (4x) and *Astr* (2x) suggests their phenotypic similarities of the traits analysed. It is clear that mono- and multiporate

taxa (species versus hybrid units) were well discriminated in the diagram.

4 Discussion

Pollen variability (Dzyuba et al. 2006; Karabournioti et al. 2007; Costa et al. 2016) can be evaluated by assessing pollen viability, morphology and pollen–stigma compatibility reactions, thus obtaining crucial information about the plant breeding systems (Kosina and Tomaszewska 2015). Pollen size (De Storme et al. 2013) and number of poruses (Kihara 1982; Kalinowski et al. 2001, 2005) are other important biological parameters for assessing the ploidy of plants, especially in newly formed hybrid units.

Size of pollen grains – Previous studies have published the lengths of the pollen grains of species and amphiploids of oat (Katsiotis and Forsberg 1995b; Meo 1999; Chrzęstek

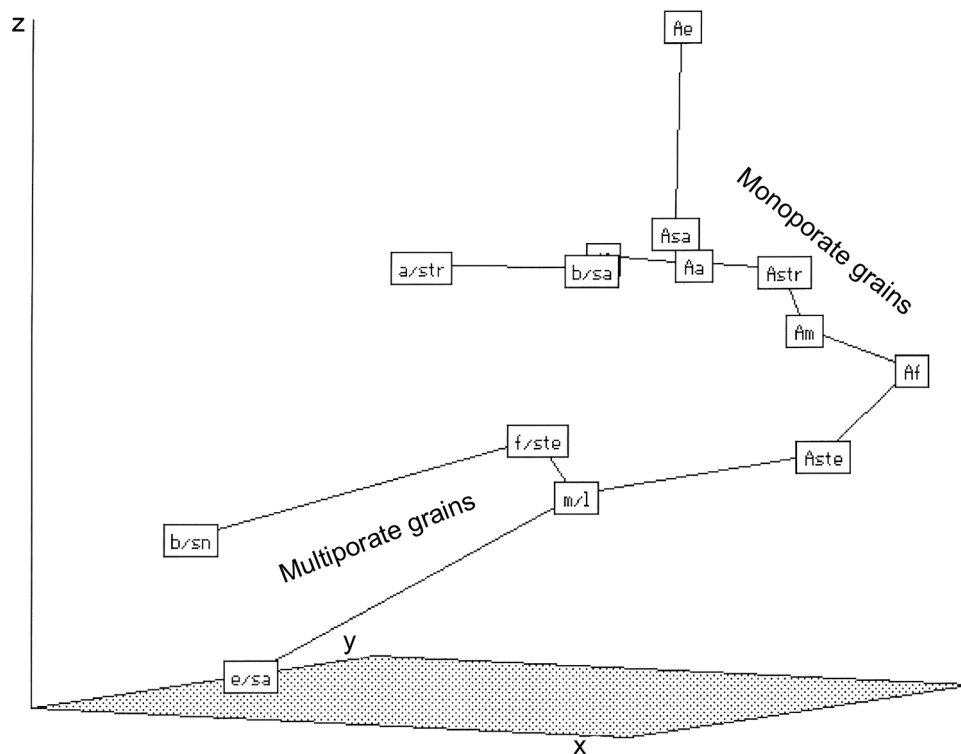


Fig. 5 Minimum spanning tree (MST) of amphiploids and parental species (Operational Taxonomic Units, OTUs) of the genus *Avena* in the ordination space (x , y and z -axes) created by using Kruskal's non-metric multidimensional scaling method (Rohlf 1994). The OTUs were described by 10 traits of pollen (viability, length, micropollens, unreduced pollen, irregular pollen, pollen with one porus, pollen with two poruses pollen with three poruses pollen with four poruses, pollen with more than four poruses). Abbreviations are given in Table 1. Two OTUs are hidden, *Ab* behind *Astr* and *Al* behind *b/sa*. The distances between the pairs of OTUs in the MST are as follows (extreme distances are in bold font): *b/sn*–*f/ste* 1.088; *f/ste*–*m/l* 0.460; *m/l*–*Aste* 0.639; *Aste*–*Af* 0.722; *Af*–*Am* 0.331; *Am*–*Ab* 0.463; ***Ab*–*Astr* 0.002**; *Astr*–*Aa* 0.231; *Aa*–*Al* 0.233; *Al*–*b/sa* 0.096, *Aa*–*Asa* 0.236; *b/sa*–*a/str* 0.505; *Asa*–*Ae* 1.112; ***m/l*–*e/sa* 1.265**

et al. 2009). The results of this study showed that, of the accessions studied, octoploid *A. eriantha* × *A. sativa* was found to have the largest pollen grains; however, hexaploid species also showed a difference in the value obtained from those published by Katsiotis and Forsberg (1995b). These differences could be due to the inter-cultivar variation or the impact of environmental conditions on the size of the pollen grains. The variation in the size of pollen between seasons confirms the impact of external factors on this feature; however, a stable pattern of inter-OTU differences was observed (see Tables 2 and 3).

The ploidy level and genotypes of both the gametophyte and the sporophyte in which the pollen grains are formed determine the size of the pollens (Ottaviano and Mulcahy 1989; Southworth and Pfahler 1992; Katsiotis and Forsberg 1995b). Our research on 15 oat accessions showed a statistically significant correlation between ploidy and the length of pollen grains. According to Katsiotis and Forsberg (1995b), the size of pollen grains was primarily determined by the ploidy level, with interspecific variation at the same level of ploidy playing a minor role. They proved that there were no significant differences in pollen size between diploid and

tetraploid accessions of oats and between hexaploids and octoploids. However, Southworth and Pfahler (1992) found that the size of pollen was influenced not only by the level of ploidy but also by the number of genomes and volume of cytoplasm, as well as interactions between these variables. The orientation of cytokineses in the microspore stem cell and the microspore—tapetum spatial relationship (Kosina and Florek 2011)—may also significantly influence the development of pollen grains in the pollen sac. In grasses, microsporocytes are arranged in a single layer that adheres to tapetum (Bhandari 1984; Batygina 1987). The equal nutritional support for microspores formed from microsporocytes after two anticlinal cytokineses is provided by such an architecture of the anther sac. A uniform mosaic of isolateral tetrads of microspores can be seen inside the anther sac when viewed from outside (Batygina 1987). However, microspores within a tetrad are not equally nourished by tapetum if cytokineses are a combination of anticlinal and periclinal events or if there are no cytokineses (Brown and Lemmon 2000; Kosina and Florek 2011). This results in the formation of microspores and pollen grains with various sizes and mating values. Thus, large, normal and small

microspores form a mosaic pattern at the walls of the anther sac. The mosaics were observed in various plant organs (Kosina 2007; Tomaszewska and Kosina 2018) as well as in anthers of *Allium senescens* subsp. *montanum* (Małecka 2008). Further, natural mutations or transgressive segregation may occur in the pollen sac of the genus *Avena*, broadening the old by new variability (Kosina 2015).

Pollen viability – Pollen grains that are able to germinate on the stigma are considered viable. A number of methods are available to determine the pollen viability, and their advantages and disadvantages have been discussed by Dafni and Firmage (2000). The authors suggested the use of several tests simultaneously, but Platje (2003) found the quick and simple in vivo pollen staining with acetocarmine to be effective.

– Factors influencing the viability of pollen grains include flower morphology, environmental conditions (temperature, humidity, soil conditions, nitrogen availability, seasonality) and indirect factors (pollen metabolism, number of nuclei, genetic conditions, breeding methods) (Dafni and Firmage 2000). Any differences in the viability of pollens between consecutive growing seasons suggest the influence of external factors on this trait (Tables 2 and 3). The results of this study showed that in one of the growing seasons, a few amphiploids and parental species showed reduced viability of pollen grains, which was most likely caused by unfavourable weather conditions such as high temperature or low air humidity. It is also known that the early stages of microspore stem cell development are particularly sensitive to high temperatures (Sakata et al. 2000). Part of the 15 oat taxa analysed in this study could have been particularly sensitive to weather conditions, thereby showing reduced viability of pollen grains in certain growing seasons.

Our studies showed a slightly lower percentage of viable pollen in oat amphiploids compared with their parental species. Warzych (2001) analysed the pollen viability in *A. fatua* and fatuoids and found a varying percentage of viable pollen between accessions, plants from one accession and between flowers located in one spikelet. Studies on the reproductive system of other grass hybrids also have provided with significant information on the viability of pollen grains. In comparison with the parental species, hybrids involving *Agrostis* L. and *Polypogon* Desf. showed a significantly lower frequency of viable pollen (Zhao et al. 2007). The average pollen viability of interspecific hybrids was higher than that of intergeneric hybrids. Further, a similar trend was shown by amphiploids resulting from the crossing of *Aegilops ovata* L. with *Secale cereale* L. (Wojciechowska and Pudelska 2002) and *Aegilops kotschyi* Boiss. and *Aegilops biuncialis* Vis. with *S. cereale* (Wojciechowska and Pudelska 2005). However, despite having distant genomes that exhibit numerous intergenomic

translocations (Tomaszewska and Kosina 2018, 2021), oat amphiploids appear to be genetically stable and meiotically compatible (Loskutov 2001). This phenomenon could be due to selection pressure directed to the chromosome number, for example, in the *A. sativa* × *Avena maroccana* Gand. (*A. maroccana* is a synonym of accepted *A. magna* H.C. Murphy & Terrell) amphidecaploid (Kushwaha et al. 2004). Loss of chromosomes over many generations and simultaneously stable cytotypes with even number of chromosomes were characteristics of amphidecaploid.

Anomalous pollen development – A reduction in the viability of pollen grains, or even complete sterility, was often due to anomalous stamen development (Leighty and Sando 1924; Protasevich 1984; Murai and Tsunewaki 1993; Kosina and Florek 2011). The results showed no anomalous stamen formation in oat accessions. Therefore, the variability observed in the viability of pollen grains between the accessions of amphiploids and their parental species might be caused by disturbances in meiosis, resulting from the coexistence of several different genomes in the hybrid nuclei (Chen et al. 1977; Tomaszewska and Kosina 2021). Meiotic anomalies can result in the formation of gametes with a reduced or increased number of chromosomes. A number of disturbances in the meiotic division, such as delayed chromosomes in telophase I and precocious chromosome segregation, were observed in interspecific and intergeneric hybrids involving such as *Agrostis* and *Polypogon* (Zhao et al. 2007). In a variety of young hybrids or amphiploids, micronuclei and formation of polyads including micropollens were observed during microsporogenesis: *Triticum aestivum* L. × *Agropyron glaucum* Roem. & Schult. (syn.) (Cicin 1978), *Triticum turgidum* L. × *Aegilops squarrosa* L. (synonym of accepted *Aegilops tauschii* Coss) (Kihara 1982) and *T. aestivum* × *Leymus mollis* (Trin.) Pilg. (Li et al. 2005). Further, in interspecific oat hybrids (Paczos-Grzęda 2003) and oat species, including diploids (Sheidai et al. 2003), disturbances in meiosis were also observed. These disturbances include chromosomes delayed in anaphase I and II and telophase I and II, micronuclei present in the microspore tetrads, bridges, chromosome elimination, multipolar systems and cytomixis. Meiotic analysis of the *Zea mays* L. inbred lines also showed similar disorders (Caetano-Pereira and Pagliarini, 2001) such as premature chromosome segregation, delayed chromosomes and micronuclei formation. Participation of such defective gametes in the process of fertilisation may contribute to the formation of an aneuploid generation with reduced fertility and viability (Zhao et al. 2007).

– A number of examples with regard to anomalous pollen grains are found in the literature. Micropollens and ultramicropollens are quite common in oats (Kosina and Florek 2011), which might form through asymmetric segregation of

chromosomes (Baptista-Giacomelli et al. 2000a). Irregular pollen grains can be formed by abnormal pollen wall formation, unfinished cytokinesis and cytomixis. An amphiploid *T. aestivum* × *L. mollis* (Li et al. 2005) showed similar disorders. Many cytomixis cases were observed in *Diplo-taxis harra* (Forssk.) Bois., where aneuploid cells showed a highly unequal number of chromosomes (Malallah and Attia 2003). Abnormal cell wall formation is one of the most common causes of large, non-reduced microspores (Ellison 1937). Anomalous cytokinesis may occur during mitosis or meiotic division I and II. Pollen binuclear stem cells were observed in the hybrid *A. barbata* × *A. strigosa* subsp. *hirtula* (Lag.) Malzev (synonym of *A. strigosa* Schreb.), which were formed due to the disturbances in cell wall formation during pre-meiotic mitosis (Holden and Mota 1956). Apoptosis occurred in the second nucleus, which was usually peripheral, and was preceded by chromatin fragmentation and nucleolus atrophy. Diploid gametes have also been observed in other species and hybrids of the genus *Avena* (Ellison 1937; Kosina and Florek 2011), and diploid gametes accounted for 1% of the pollen grains in tetraploid *Avena vaviloviana* Malzev (Mordv.) (Katsiotis and Forsberg 1995a). Large, unreduced microspores can be formed by cytomixis or restitution of nuclei during meiosis (Stuczyński et al. 1994). Due to the delayed growth of their thick pollen tubes, unreduced pollen grains show low mating value and can be inefficient in the fertilisation process, as evidenced in *Prunus spinosa* L. (Staszak 2004).

Number of poruses – Porus morphogenesis appears to be related to de novo synthesis of callose in a microspore (Teng et al. 2005). The pattern of callose deposition in a differentiating pollen grain appeared to be different in grasses. In *Brachypodium phoenicoides* (L.) Roem. & Schult. (Kłyk 2005), scattered lenses of callose adjacent to intine were observed, whereas in *A. fatua*, callose was synthesised around a pore and disappeared towards the opposite pole (Warzych 2001).

– The number of poruses in the pollen wall, through which pollen tubes can germinate, varies between species. Pollens with varying number of apertures are frequently seen in the same species, including *Viola diversifolia* (DC.) W. Becker (Dajoz et al. 1991), *Viola arvensis* Murray and *Nicotiana tabacum* L. (Ressayre et al. 1998). The grass variety is characterised by single-porate pollen grains; therefore, pollen with a higher number of poruses should be considered anomalous. The results of this study showed that all amphiploids examined produced multiporate pollen grains, whereas their parental species produced pollen with only one porus. Multiporate pollen grains were reported in different hybrids and amphiploids of grasses—diporate grains were developed in an F1 hybrid generation of *T. turgidum* × *Ae. squarrosa* (Kihara 1982); half of the pollen pool of *Miscanthus*

Anderss. ‘Giganteus’ had two to five poruses (Linde-Laursen 1993); pollen with 10 poruses has been reported in an amphiploid *T. aestivum* × *L. mollis* (Li et al. 2005). It should be emphasised that multiporate pollen grains can germinate through many pores. As a result, there arises a competition between the tubes during their growth, thereby significantly reducing their effectiveness in the fertilisation process. Such a case was observed in the amphiploid *A. barbata* × *A. sativa* subsp. *nuda* (Florek 2013; Kosina et al. 2014).

– The increase in the number of poruses might be caused by the independent activity of genes located in different genomes (Kalinowski et al. 2001, 2005). However, four poruses were observed in unreduced polyploid monads in *Triticum aestivum* L. Their origin was related to four monoporate microspores. The proliferation of poruses seems to be correlated with a multipolar divisional spindle, but many poruses differentiated also at its lack (Dover 1972). Therefore, plants formed by the process of polyploidisation are often characterised by the presence of multiporate pollen grains. Few authors have discussed the relationship between apomictic mode of reproduction and multiporate pollen. Liu et al. (2004) recorded an association between multiporate pollen grains and apomictic type of reproduction in *Leptochloa panicea* (Retz.) Ohwi and *Schmidtia pappophoroides* Steud. ex J.A. Schmidt. Genera *Apluda* L., *Heteropogon* Pers., *Panicum* L., *Paspalum* L., *Pennisetum* Rich. (Ma et al. 2009) and *Bothriochloa* Kuntze (Ma and Huang 2007) are other apomicts, among grasses, that produce multiporate pollens. All of these genera include species showing high levels of ploidy (Higgins et al. 2021; Tomaszewska et al. 2021a, b). It can be assumed that formation of multiporate pollen is associated with young allopolyploids and the interaction of various genomes in their nuclei, whereas apomictic reproduction allows the population to function with a generative defect. A number of disturbances in the process of micro- and macrosporogenesis are observed in allopolyploids, which can result in apomixis. Cytogenetic analyses have revealed disturbed chromosome segregation and delayed chromosomes in *Bothriochloa ischaemum* (L.) Keng (Ma and Huang 2007). Due to sterility of pollen in *Calamagrostis hakonensis* Franch. & Sav. and *Dichanthium aristatum* (Poir.) C.E. Hubb (Ma et al. 2009), double fertilisation was impossible, so apomixis was considered to be an alternative to sexual reproduction. It was found that plant populations with higher numbers of chromosomes, which are prone to meiotic disorders and defective pollens, showed a transition from generative reproduction to apomixes (Grant 1981). Therefore, more research is needed to understand the interaction between the development of multiporate pollen grains and apomictic reproduction and to explore the cause and effect.

– In conclusion, the study revealed that the pollen of the oat species and amphiploids obtained from their crosses

showed high viability, which enables generative reproduction and ultimately the proper development of caryopses. However, the per cent of pollen viability in amphiploids was significantly lower than that in species. In addition, amphiploid flowers produced a low percentage of anomalous pollens. The results also showed variations in the size of pollen grains during the growing seasons, but a stable interrelations between the taxa. The viability of pollen grains was more significantly affected by environmental factors compared with the length of pollen grain, and no correlation was found between the viability of pollens and variability in grain sizes. In the ordination space, amphiploids were discriminated from parental species. In both groups, a positive correlation between the pollen size and the level of ploidy was stable; however, in the cloud of correlation points along the regression line, amphiploids were located among species with a high level of ploidy and were extreme units there. Pollen morphotypes showed a low frequency of developmental anomalies, with the formation of pollen micrograins being the most common. This proved that some pollen grains were chromosomally unbalanced and were formed in polyads. Anomalies were more common in the hybrid types, including the formation of pollen grains with many poruses that occurred only in amphiploids. The frequency of multiporate grains was strongly correlated with the frequency of micropollens. This correlation can show that multiporate- and micro-cells were of multipolar-spindle cells origin. Due to their germination with many pollen tubes and mutual competition, multiporate grains will have a lower rate of reproductive success (Florek 2013; Kosina et al. 2014). Monoporate types (species) were well discriminated from multiporate types (amphiploids) in the ordination space. The findings revealed that pollen viability was high in amphiploids, which would prove their genomic/chromosomal stability achieved through directional selection across many generations of reproduction in life collections.

Acknowledgements We are grateful to Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Braunschweig, Germany; National Small Grains Collection, Aberdeen, Idaho, USA; and Vavilov Institute of Plant Industry, St. Petersburg, Russia for their generous provision of seeds.

Author contribution PT designed and conducted all analyses and interpreted the results. PT and RK prepared figures and wrote the article. RK provided research idea, supervised the experiments and research documentation. The authors read and approved the manuscript.

Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The authors guarantee compliance with ethical standards.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000a) Elimination of micronuclei from microspores in a Brazilian oat (*Avena sativa* L.) variety. *Genet Mol Biol* 23:681–684
- Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000b) Meiotic behaviour in several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia* 65:371–378
- Batygina TB (1987) Chlebnoe zerno. Atlas. Nauka, Leningrad
- Bhandari NN (1984) The microsporangium. In: Johri BM (ed) *Embryology of Angiosperms*. Springer, Berlin, Heidelberg
- Brown RC, Lemmon BE (2000) The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae). *Amer J Bot* 87:1–11
- Caetano-Pereira CM, Pagliarini MS (2001) A new meiotic abnormality in *Zea mays*: multiple spindles associated with abnormal cytokinesis in both divisions. *Genome* 44:856–871
- Chen C, Qualset CO, Stanford RH (1977) Meiotic studies of secondary 42-chromosome *Triticales*. *Bot Bull Acad Sinica* 18:89–99
- Chrzastek M, Kruk K, Okoń S, Wojtowicz E (2009) Wpływ formy ojcowskiej na żywotność pyłku i niektóre cechy plonotwórcze mieszańców międzygatunkowych *A. sativa* L. cv. Borowiak x *Avena sterilis* L. *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin* 252:245–253
- Costa J, Castro S, Loureiro J, Barrett SCH (2016) Experimental insights on the function of ancillary pollen and stigma polymorphisms in plants with heteromorphic incompatibility. *Evolution* 71:121–134
- Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Syst Evol* 222:113–132
- Dajoz I, Till-Bottraud I, Gouyon P-H (1991) Evolution of pollen morphology. *Science* 253:66–68
- De Storme N, Zamariola L, Mau M, Sharbel F, Geelen D (2013) Volume-based pollen size analysis: an advanced method to assess somatic and gametophytic ploidy in flowering plants. *Plant Reprod* 26:65–81

- Dover GA (1972) The organization and polarity of pollen mother cells of *Triticum aestivum*. *J Cell Sci* 11:699–711
- Dzyuba OF, Shurekova OV, Tokarev PI (2006) On the natural polymorphism of pollen grains of *Acer tataricum* L. *Paleontolog J* 40:S590
- Elliot AC (1994) KWIKSTAT 4- statistical data analysis program. Texassoft, Cedar Hill
- Ellison W (1937) Polyploid gamete formation in diploid *Avena* hybrids. *J Genet* 34:287–295
- Florek M (2013) SSZmienność strukturalna i cytogenetyczna amfiploida *Avena barbata* × *Avena nuda* o genomach natywnych i demetylowanych. Dissertation, University of Wrocław
- Grant V (1981) Plant speciation. Columbia University Press, New York
- Higgins J, Tomaszewska P, Pellny T K, Castiblanco V, Arango J, Tohme J, Schwarzacher T, Mitchell RA, Heslop-Harrison JS, de Vega JJ (2021) The five *Urochloa* spp. used in development of tropical forage cultivars originate from defined subpopulations with differentiated gene pools. *BioRxiv*. <https://doi.org/10.1101/2021.07.21.453213>
- Holden JWH, Mota M (1956) Non-synchronised meiosis in binucleate pollen mother cells of an *Avena* hybrid. *Heredity* 10:109–117
- Kalinowski A, Winiarczyk K, Wojciechowska B (2001) Pollen proteins after two-dimensional gel electrophoresis and pollen morphology of the amphiploids *Aegilops kotschy* and *Ae. variabilis* with *Secale cereale*. *Sex Plant Reprod* 14:153–161
- Kalinowski A, Klimko M, Wojciechowska B (2005) Pollen morphology and two-dimensional patterns of pollen coat and protoplast proteins in *Aegilops kotschy* × *Secale cereale* amphiploids. *Acta Biol Cracov Bot* 47:97–110
- Karabournioti S, Eleftheriou EP, Thrasyvoulou A, Fasseas C (2007) Pollen polymorphism in *Thymus capitatus* (Lamiaceae). *Botany* 85:493–500
- Katsiotis A, Forsberg RA (1995a) Discovery of $2n$ gametes in tetraploid oat *Avena vaviloviana*. *Euphytica* 81:1–6
- Katsiotis A, Forsberg RA (1995b) Pollen grain size in four ploidy levels of genus *Avena*. *Euphytica* 83:103–108
- Kihara H (1982) Wheat studies – retrospect and prospects. *Developments in crop science* 3. Kodansha Ltd., Tokyo
- Kłyk B (2005) Zmienność mikrostrukturalna niektórych gatunków rodzaju *Brachypodium* P. Beauv. Dissertation, University of Wrocław
- Kosina R (2015) Variation of reproduction in some species of the tribe Aveneae (Poaceae). *Modern Phytomorphology* 8:11–14
- Kosina R, Tomaszewska P (2015) Variability of breeding system, caryopsis microstructure and germination in annual and perennial species of the genus *Brachypodium* P. Beauv *Genet Resour Crop Ev* 63:1003–1021
- Kosina R, Florek M, Tomaszewska P (2014) Pollen grain morphogenesis in Triticeae and *Avena* amphiploids. *Ann Wheat Newslet* 60:102–103
- Kosina R, Florek M (2011) On some characteristics of *Avena* (Poaceae) pollen grains. In: Frey L (ed) *Advances in grass biosystematics*. W. Szafer Institute of Botany, Polish Academy of Science, Kraków, pp 107–117.
- Kosina R (2007) Some topics of the grass mosaics. In: Frey L (ed) *Biological issues in grasses*. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp 159–167
- Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29:1–27
- Kushwaha N, Zadoo SN, Choubey RN (2004) Breakdown of polyploidy and isolation of stable polysomics in amphidecaploid *Avena sativa* L. × *A. maroccana* Gdgr. *Cytologia* 69:41–47
- Leighty CE, Sando WJ (1924) Pistillody in wheat flowers. *J Hered* 15:263–268. <https://doi.org/10.1093/oxfordjournals.jhered.a102468>
- Li X-F, Liu S-B, Gao J-R, Lu W-H, Wang H-G (2005) Abnormal pollen development of bread wheat-*Leymus mollis* partial amphiploid. *Euphytica* 144:247–253
- Linde-Laursen I (1993) Cytogenetic analysis of *Miscanthus* ‘Giganteus’, an interspecific hybrid. *Hereditas* 119:297–300
- Linde-Laursen I, von Bothmer R (1993) Aberrant meiotic divisions in a *Hordeum lechleri* × *H. vulgare* hybrid. *Hereditas* 118:145–153
- Liu Q, Zhao N-X, Hao G (2004) Pollen morphology of the Chloridoideae (Gramineae). *Grana* 43:238–248
- Loskutov IG (2001) Interspecific crosses in the genus *Avena* L. *Russ J Genet* 37:467–475
- Ma GH, Huang XL (2007) Cytological and embryological studies on apospory in *Bothriochloa ischaemum* L. *Acta Biol Hung* 58:421–429
- Ma G, Huang X, Xu Q, Bunn E (2009) Multiporate pollen and apomixis in Panicoideae. *Pak J Bot* 41:2073–2082
- Malallah GA, Attia TA (2003) Cytomixis and its possible evolutionary role in a Kuwaiti population of *Diptotaxis harra* (Brassicaceae). *Bot J Linn Soc* 143:169–175
- Małeczka A (2008) Mejoza u *Allium senescens* subsp. *montanum*. dissertation, University of Wrocław
- Mendes-Bonato AB, Junqueira Filho RG, Pagliarini MS, de Valle CB, de Oliveira Penteadó MI (2002) Unusual cytological patterns of microsporogenesis in *Brachiaria decumbens*: abnormalities in spindle and defective cytokinesis causing precocious cellularization. *Cell Biol Int* 26:641–646
- Meo AA (1999) Impact of pollen and intergenetic crosses between graminaceous (Poaceae) plants. *Pak J Biol Sci* 2:809–812
- Mujeeb KA, Thomas JB, Rodriguez RR, Waters RF, Bates LS (1978) Chromosome instability in hybrids of *Hordeum vulgare* L. with *Triticum turgidum* and *T. aestivum*. *J Hered* 69:179–182. <https://doi.org/10.1093/oxfordjournals.jhered.a108920>
- Murai K, Tsunewaki K (1993) Photoperiod-sensitive cytoplasmic male sterility in wheat with *Aegilops crassa* cytoplasm. *Euphytica* 67:41–48
- Ottaviano E, Mulcahy DL (1989) Genetics of Angiosperm pollen. *Adv Genet* 26:1–64
- Paczos-Grzęda E (2003) Badania cytogenetyczne i molekularne mieszańców międzygatunkowych heksaploidalnego owsa *Avena sativa* L. × *Avena sterilis* L. oraz form wyjściowych. *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin* 229:21–32
- Platje J (2003) Charakterystyka cytogenetyczna niektórych taksonów rodzaju *Magnolia* L. Dissertation, University of Wrocław
- Protasevich RT (1984) Morfologo-anatomičeskie osobennosti rastenij s mužskoj steril’nost’ju. *Nauka i Technika*, Minsk
- Quarin CL, Espinoza F, Martinez EJ, Pessino SC, Bovo OA (2001) A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sex Plant Reprod* 13:243–249. <https://doi.org/10.1007/s004970100070>
- Ressayre A, Godelle B, Mignot A, Gouyon PH (1998) A morphogenetic model for pollen aperture pattern in flowering plants. *J Theor Biol* 193:321–334
- Richards AJ (1986) Plant breeding systems. George Allen & Unwin, London
- Rohlf FJ (1981) Spatial representation of phylogenetic trees computed from dissimilarity matrices. *Int. Symp. on Concept and Method in Paleontology Barcelona* pp 303–311
- Rohlf FJ (1994) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.80. Exeter Software, New York
- Sakata T, Takahashi H, Nishiyama I, Higashitani A (2000) Effects of high temperature on the development of pollen mother cells and microspores in barley *Hordeum vulgare* L. *J Plant Res* 113:395–402
- Sheidai M, Koobaz P, Zehzad B (2003) Meiotic studies of some *Avena* species and populations in Iran. *J Sci, Islam Rep Iran* 14:121–131

- Southworth D, Pfahler P (1992) The effects of genotype and ploidy level on pollen surface sculpturing in maize (*Zea mays* L.). *Amer J Bot* 79:1418–1422
- Staszak A (2004) Morfometria *Prunus spinosa* L. z populacji okolic Wrocławia. Dissertation, University of Wrocław
- Stuczyński M, Stuczyńska J, Stuczyńska E (1994) Niezredukowane gamety w hodowli traw. *Genet Pol* 35A:3–9
- Teng N, Huang Z, Mu X, Jin B, Hu Y, Lin J (2005) Microsporogenesis and pollen development in *Leymus chinensis* with emphasis on dynamic changes in callose deposition. *Flora* 200:256–263
- Thomas H, Peregrine WTH (1964) Chromosome mosaics in synthetic amphiploids in the Avenae. *Chromosoma* 15:123–131
- Tomaszewska P, Kosina R (2018) Instability of endosperm development in amphiploids and their parental species in the genus *Avena* L. *Plant Cell Rep* 37:1–14
- Tomaszewska P, Kosina R (2021) Cytogenetic events in the endosperm of amphiploid *Avena magna* x *A. longiglumis*. *J Plant Res* 134:1047–1060. <https://doi.org/10.1007/s10265-021-01314-3>
- Tomaszewska P, Pellny TK, Hernández LM, Mitchell RAC, Castiblanco V, De Vega JJ, Schwarzacher T, Heslop-Harrison JS (2021) Flow cytometry-based determination of ploidy from dried leaf specimens in genomically complex collections of the tropical forage grass *Urochloa* s.l. *Genes* 12:957
- Tomaszewska P, Vorontsova M, Renvoize SA, Ficinski SZ, Tohme J, Schwarzacher T, Castiblanco V, De Vega JJ, Mitchell RAC, Heslop-Harrison JS (2021b) Complex polyploid and hybrid species in an apomictic and sexual tropical forage grass group: genomic composition and evolution in *Urochloa* (*Brachiaria*) species. <https://doi.org/10.1101/2021b.02.19.431966>
- Warzych A (2001) Zmienność mikrostrukturalna *Avena fatua* L. i taksonów pokrewnych. Dissertation, University of Wrocław
- Wojciechowska B, Pudelska H (2002) Hybrids and amphiploids of *Aegilops ovata* L. with *Secale cereale* L.: production, morphology and fertility. *J Appl Genet* 43:415–421
- Wojciechowska B, Pudelska H (2005) Production and characterization of amphiploids of *Aegilops kotschy* and *Ae. biuncialis* with *Secale cereale*, and of backcross hybrids of *Ae. biuncialis* × *S. cereale* amphiploids with 2x and 4x *S. cereale*. *J Appl Genet* 46:157–161
- Zhao H, Bughrara SS, Wang Y (2007) Cytology and pollen grain fertility in creeping bentgrass interspecific and intergeneric hybrids. *Euphytica* 156:227–235

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.