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POLLEN FERTILITY ASSESSMENT THROUGH ACETOCARMINE STAINING AND *IN VITRO* GERMINATION IN *SOLANUM TUBEROSUM* L.

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SUMMARY

The wide occurrence of potato genotypes characterized by male sterility considerably complicates the realization of breeding programs. Therefore, male fertility in potato germplasm requires assessment as an important task. In the fresh study, 57 breeding lines from the Russian Potato Research Center and 85 cultivars of different origins underwent acetocarmine staining and *in vitro* germination assessment. With acetocarmine staining, 22 samples exhibited with tetrad sterility. In the remaining 120 samples, stained pollens vary from 0% to 88.7%, and germination varies from 0% to 19.4%. The pollen fertility assessment data obtained through acetocarmine staining and *in vitro* germination differed significantly. In the studied potato accessions, acetocarmine staining provided 18.3% sterile samples, while 50.7% by *in vitro* germination. The highest stained pollens (>80%) appeared in five potato accessions, i.e., 3004-7 (88.7%), 3000-32 (86.5%), 21.32-1 (81.8%), 17.32-1 (80.3%), and cultivar Garant (86.8%). In most potato accessions, the colored pollen grains ranged from 40% to 70%. The maximum percent of pollen germination occurred in the sample, 21.32-1 (19.4%). However, seven accessions (21.32-1, 18.5-17, 20.3-6, 20.33-8, and cultivars Edison, Queen Anne, and Garant) showed more than 10% pollen germination. During the growing season of 2022, the weather was hotter and drier compared with past long-term data, which could affect a decrease in the ability of pollens to germinate. The obtained results on male fertility will help in the study of the genetic control of pollen fertility and a further improvement in the breeding of *Solanum tuberosum* L.

Keywords: Potato (*Solanum tuberosum* L.), pollen fertility, male sterility, acetocarmine staining, *in vitro* germination, breeding techniques

Key findings: The results showed significant differences among the potato samples for pollen fertility. The 15.5% potato accessions characterized by the presence of tetrads were completely sterile. The pollen fertility assessment data obtained through acetocarmine staining and *in vitro* germination differed significantly. However, the acetocarmine staining can serve more useful for preliminary screening and validation of *Solanum tuberosum* accessions with tetrad sterility.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world and its accelerated breeding could significantly contribute to solving of the global food security issue. A known fact is that potato forms characterized by male sterility significantly exist commonly in cultured tetraploids (Salaman, 1910; Stout and Clark, 1924; Fukada, 1925; Ellison, 1936; Fineman, 1947). Potato has six basic types of cytoplasm (A, M, P, T, W, and D), and three of them (T, W, and D) link with male sterility (Hosaka and Sanetomo, 2012), which prevail in the current gene pool of cultivated potatoes (Sanetomo and Gebhardt, 2015; Gavrilenko *et al*., 2019). In addition, recognized reports also stated that the presence of nuclear genes in potato accessions contributes to the genetic control of pollen fertility (Iwanaga *et al*., 1991; Ortiz *et al*., 1993).

The prevalence of male sterility in potatoes significantly complicates the management of breeding programs. In recent years, a new direction in modern potato breeding based on the creation of diploid hybrids through heterosis, including potato reproduction using true botanical seeds, has intensively developed (Lindhout *et al*., 2011; Jansky *et al*., 2016). In this regard, the study of complex signs associated with the generative reproduction of potatoes has gained great significance. Therefore, the male fertility assessment in potato accessions proves an important task in modern breeding. Despite a wide range of works devoted to studying morphological and chemical features of pollen grains of cultivated plants (Kumar *et al*., 2015; Azka *et al*., 2021; Horčinová-Sedláčková *et al*., 2021; Zubkova *et al*., 2022), there exists a limited number of tools for the estimation of pollen fertility and viability.

Acetocarmine glycerol jelly is one of the most common stain techniques for potato pollen research. This method is fast and cheap and allows to distinguish viable pollen with integral cytoplasm by bright red color (Ordoñez, 2014). The simplicity of this method makes it accessible for the analysis of a large number of samples, but like other vital stain methods, it can also show false results about cytoplasm presence in pollens (Dafni and Firmage, 2000). Trognitz (1991) findings showed that this method gives the least accurate results for assessing pollen fertility in potatoes. Another popular method for evaluating pollen fertility is the *in vitro* pollen germination method, which is quite simple and fast and also suitable for analyzing a large number of selections. This method showed a correlation between fruit-set and seed-set in many species (Visser, 1955). On the other side, it showed more susceptibility to environmental conditions (Stanley and Linskens, 1974). Therefore, it seemed interesting to perform a comparative assessment of the pollen fertility of potato samples by two different complementary methods - acetocarmine staining and *in vitro* germination. Taking into account the influence of weather conditions on the results, an assessment of the weather conditions and a comparison with long-term past data also proceeded.

Breeding samples from the collection of Lorch Potato Research Center, Russian Federation, as well as, potato cultivars of various origins, were used for this study. Lorch Potato Research Center is the largest potato breeding center in the Russian Federation. The Institute maintains a collection of potato samples that are sources and donors of valuable traits in breeding programs. Obtaining new data on male fertility of samples will allow further improvement of breeding programs and contribute to the study of genetic control of pollen fertility.

MATERIALS AND METHODS

Plant material

The study included 57 breeding lines from the collection of the Potato Research Center and 85 cultivars of different origins (Table 1). All the potato samples grown in 2022 ensued in the field at the experimental base in Korenevo, Moscow region, Russia. Potato sample selection proceeded during the flowering period, from 26 July 26 to 10 August 2022.

Acetocarmine staining

Fully dried anthers placed against a vibrating device (an electric toothbrush without a replaceable head) extracted the pollen above a clean glass slide. After each potato flower sample, cleaning the device used 96% ethanol. Acetocarmine glycerol jelly (45% acetic acid boiled with 2% carmine and then filtered and mixed with glycerol in a ratio of 1:1) was prepared and placed on glass slides to stain the pollen grain samples, as described by Ordoñez (2014). Staining took for 3-5 min before studying the samples under a light microscope with 200×–400× magnification.

Table 1. Accessions of *Solanum tuberosum* and pollen fertility data based on acetocarmine staining and *in vitro* germination.

No.	Accession	Country of origin	Stained pollen (%)	Germinated pollen (%)	Pollen tetrads	Germinated pollens out of stained pollen (%)
1	Akrosiya	Russia	31.2	0.0	no	0.0
2	Alwara	Germany	0.0	0.0	yes	NA
3	Alyaska	Russia	3.2	0.0	no	0.0
4	Alyj parus	Russia	64.6	6.1	no	9.4
5	Arielle	The Netherlands	45.1	0.0	no	0.0
6	Austin	Germany	54.1	8.2	no	15.2
7	Azhur	Russia	3.0	0.0	no	0.0
8	Babushka	Russia	39.9	0.0	no	0.0
9	Bernina	Germany	0.0	0.0	yes	NA
10	Bryanskij	Russia	20.9	<1	no	NA
11	Bryanskij krasnyj	Russia	0.0	0.0	yes	NA
12	Buket	Russia	47.7	0.0	no	0.0
13	Bylina Sibiri	Russia	29.2	0.0	no	0.0
14	Chajka	Russia	0.5	0.0	no	0.0
15	Chugunka	Russia	0.7	0.0	no	0.0
16	Colomba	The Netherlands	28.2	0.0	no	0.0
17	Dachnyj	Russia	47.4	<1	no	NA
18	Delphine	Germany	0.0	0.0	yes	NA
19	Dounia	Russia	58.9	<1	no	NA
20	Druid	Ireland	8.2	0.0	no	0.0
21	Edison	Germany	74.9	13.6	no	18.1
22	Eliksir	Russia	13.5	0.0	no	0.0
23	Estrella	Germany	0.0	0.0	yes	NA
24	Eurostarch	Germany	0.0	0.0	yes	NA
25	Evgeniya	Russia	60.2	2.0	no	3.3
26	Ferrari	France	54.0	2.3	no	4.2
27	Flamingo	Russia	29.5	<1	no	NA
28	Fresko	The Netherlands	66.4	<1	no	NA
29	Garant	Belarus	86.8	11.6	no	13.4
30	Garantiya	Russia	0.0	0.0	yes	NA
31	Golubizna	Russia	14.5	0.0	no	0.0
32	Gubernator	Russia	35.5	6.6	no	18.6
33	Hibinskij dvuhurozhajnyj	Russia	0.0	0.0	no	NA
34	Ipatovskij	Russia	34.5	<1	no	NA
35	Irbitskij	Russia	57.7	<1	no	NA
36	Juwel	Germany	11.9	3.3	no	27.8
37	Kalinka	Russia	58.8	0.0	no	0.0
38	Karmen	Russia	41.8	<1	no	NA
39	Kazachok	Russia	11.6	0.0	no	0.0
40	Kingsman	UK	25.2	0.0	no	0.0
41	Kiwi	Russia	40.0	0.0	no	0.0
42	Krasa Meshchery	Russia	42.9	<1	no	NA
43	Krone	Germany	14.1	0.0	no	0.0
44	Kuzovok	Russia	45.5	9.8	no	21.6
45	Lel'	Belarus	64.5	0.0	no	0.0
46	Lionheart	UK	2.3	0.0	no	0.0
47	Liseta	The Netherlands	26.1	<1	no	NA
48	Magadanskij	Russia	72.5	1.7	no	2.4
49	Malinovka	Russia	0.0	0.0	yes	NA
50	Mandola	France	47.6	<1	no	NA
51	Merlot	Germany	26.0	7.2	no	27.7
52	Mondeo	The Netherlands	72.8	1.3	no	1.8
53	Musinskij	Russia	50.3	6.7	no	13.4
54	Nadezhda	Russia	0.0	0.0	no	NA
55	Nart	Russia	47.1	4.4	no	9.4
56	Nayada	Russia	28.5	9.4	no	32.9
57	New York	USA	46.9	5.4	no	11.5
58	Olimp	Russia	65.5	0.0	no	0.0
59	Omega	Germany	51.1	1.5	no	3.0
60	Opal	Germany	58.0	6.3	no	10.8

Table 1. (cont'd).

No.	Accession	Country of origin	Stained pollen (%)	Germinated pollen (%)	Pollen tetrads	Germinated pollens out of stained pollen (%)
61	Orlak	Russia	12.2	0.0	no	0.0
62	Petrovich	Russia	48.0	8.0	no	16.6
63	Pobeda	Russia	32.5	<1	no	NA
64	Prinz	Russia	0.0	0.0	no	NA
65	Purshenskij	Russia	37.1	8.6	no	23.2
66	Queen Anne	Germany	79.0	12.5	no	15.9
67	Red Fantasy	Germany	70.9	7.8	no	11.0
68	Ricarda	Germany	71.8	5.0	no	6.9
69	Roko	Austria	0.0	0.0	yes	NA
70	Sadon	Russia	50.0	<1	no	NA
71	Samba	Russia	43.7	3.9	no	8.8
72	Sante	The Netherlands	51.5	0.0	no	0.0
73	Serdolik	Russia	0.0	0.0	yes	NA
74	Sevim	Germany	35.5	0.0	no	0.0
75	Sierra	UK	74.1	0.0	no	0.0
76	Solncecvet	Russia	0.0	0.0	yes	NA
77	Sultan	Russia	7.0	<1	no	NA
78	Tarasov	Russia	18.2	8.6	no	47.3
79	Verdi	Germany	0.0	0.0	yes	NA
80	Volat	Belarus	17.6	0.0	no	0.0
81	Zagadka	Ukraine	6.5	0.0	no	0.0
82	Zhivica	Belarus	0.0	0.0	yes	NA
83	Zhuravinka	Belarus	14.3	0.0	no	0.0
84	Zol'skij	Russia	0.0	0.0	no	NA
85	Zumba	Russia	29.3	0.0	no	0.0
86	2727-29	Russia	0.0	0.0	yes	NA
87	1-16-4	Russia	0.0	0.0	yes	NA
88	1198	Russia	67.1	5.5	no	8.2
89	1026	Russia	29.4	2.1	no	7.3
90	18.27-17	Russia	0.0	0.0	yes	NA
91	1200	Russia	39.6	<1	no	NA
92	48-6	Russia	0.0	0.0	yes	NA
93	680-1-21	Russia	10.3	<1	no	NA
94	16.17-44	Russia	43.5	0.0	no	0.0
95	62-1	Russia	56.0	8.6	no	15.3
96	905;92x	Russia	42.3	0.0	no	0.0
97	3000-32	Russia	86.5	4.1	no	4.8
98	3000-33	Russia	65.2	8.8	no	13.5
99	3000-37	Russia	37.4	<1	no	NA
100	3004-7	Russia	88.7	6.6	no	7.4
101	323-1	Russia	68.0	1.5	no	2.1
102	19.23-3	Russia	12.8	<1	no	NA
103	12.14-10	Russia	0.0	0.0	yes	NA
104	20.4-13	Russia	27.7	0.0	no	0.0
105	20.6-7	Russia	41.9	5.9	no	14.1
106	18.5-17	Russia	54.3	14.4	no	26.5
107	20.3–4	Russia	0.0	0.0	no	NA
108	20.17-7	Russia	44.7	8.7	no	19.4
109	20.34-2	Russia	0.0	0.0	yes	NA
110	19.14-15	Russia	32.2	7.5	no	23.2
111	21.32-1	Russia	81.8	19.4	no	23.7
112	20.33-3	Russia	56.7	8.5	no	14.9
113	20.3-5	Russia	52.0	0.0	no	0.0
114	20.15-17	Russia	47.0	4.9	no	10.4
115	20.33-6	Russia	74.8	0.0	no	0.0
116	18.18-10	Russia	20.0	0.0	no	0.0
117	20.33-4	Russia	50.9	0.0	no	0.0
118	20.3-6	Russia	58.3	12.6	no	21.6
119	18.10-7	Russia	18.5	0.0	no	0.0
120	21.32-2	Russia	66.9	8.5	no	12.7

Table 1. (cont'd).

No.	Accession	Country of origin	Stained pollen (%)	Germinated pollen (%)	Pollen tetrads	Germinated pollens out of stained pollen (%)
121	19.38-2	Russia	33.7	0.0	no	0.0
122	15.3-26	Russia	36.5	0.0	no	0.0
123	20.3-2	Russia	37.5	0.0	no	0.0
124	47.04-21	Russia	0.0	0.0	yes	NA
125	17.32-1	Russia	80.3	<1	no	NA
126	20.33-7	Russia	76.7	4.1	no	5.3
127	19.24-6	Russia	71.8	9.3	no	13.0
128	13.5-7	Russia	20.9	4.6	no	22.2
129	19.16-4	Russia	32.9	0.0	no	0.0
130	99.6-10	Russia	34.6	0.0	no	0.0
131	20.33-8	Russia	57.1	10.9	no	19.0
132	20.3-3	Russia	48.9	4.1	no	8.4
133	19.12-3	Russia	72.3	0.0	no	0.0
134	15.3-36	Russia	17.1	0.0	no	0.0
135	16.28-8	Russia	29.0	3.7	no	12.8
136	21.2-6	Russia	0.0	0.0	yes	NA
137	18.14-17	Russia	8.7	0.0	no	0.0
138	20.34-19	Russia	0.0	0.0	no	NA
139	16.24-1	Russia	36.3	5.2	no	14.4
140	16.30-8	Russia	28.5	<1	no	NA
141	46.98-6	Russia	13.0	0.0	no	0.0
142	16.20-11	Russia	0.0	0.0	yes	NA

Pollen grains with a bright red stained cytoplasm and in a circle shape got marked as viable, with the deformed and non-stained pollen grains classified as sterile. Ten randomly chosen observation fields (with 30-80 pollen grains on each field) on each slide received accounting, with all the stained and non-stained pollen grains on those fields also counted. Overall, examining each sample covered not less than 300 pollen grains.

In vitro pollen germination

Preparing the culture medium for pollen grains germination progressed with 20 g of sucrose and 0.2 ml of Tween-20 mixed with 100 ml of distilled water, adding boric acid to the mixture until reaching a pH value of 5.5 (Ordoñez, 2014). The extraction of pollen grains used fully dried anthers placed against a vibrating device above a sterile plastic petri dish with 3-4 drops of sucrose-based germination culture medium. Pollen grains were gently mixed in the dish with a clean wooden toothpick and left overnight at room temperature in a humidity chamber (placing a piece of filter paper moistened with distilled water on the cap of each petri dish). After 14-18 h, the sample examination under a light microscope followed by 200× magnification (stained with iodine-potassium for 5 min before examination). A pollen grain successfully germinated when the pollinic tube length was greater than the diameter of the pollen grain. Ten randomly chosen observation fields on each dish gained accounting, with all germinated and non-germinated pollen grains counted. Each experiment had two repetitions.

Weather conditions

Data collection on air temperature and precipitation continued, from 1 May to 31 August 2022, at the Weather Station, Lorch Potato Research Center, Korenevo, Moscow region, Russia. Data for the same period, from 2001–2019 were also used for comparison. Based on daily indicators, average indicators for days were calculated and charts were made based on the results.

RESULTS

Acetocarmine staining

According to the acetocarmine analysis results, 22 potato samples exhibited with tetrad sterility, including nine (15.8%) samples of the breeding collection of Lorch Potato Research Center, Korenevo, Moscow region, Russia, and 13 cultivars (15.3% of the total number of analyzed cultivars from other regions) (Figures 1 and 2).

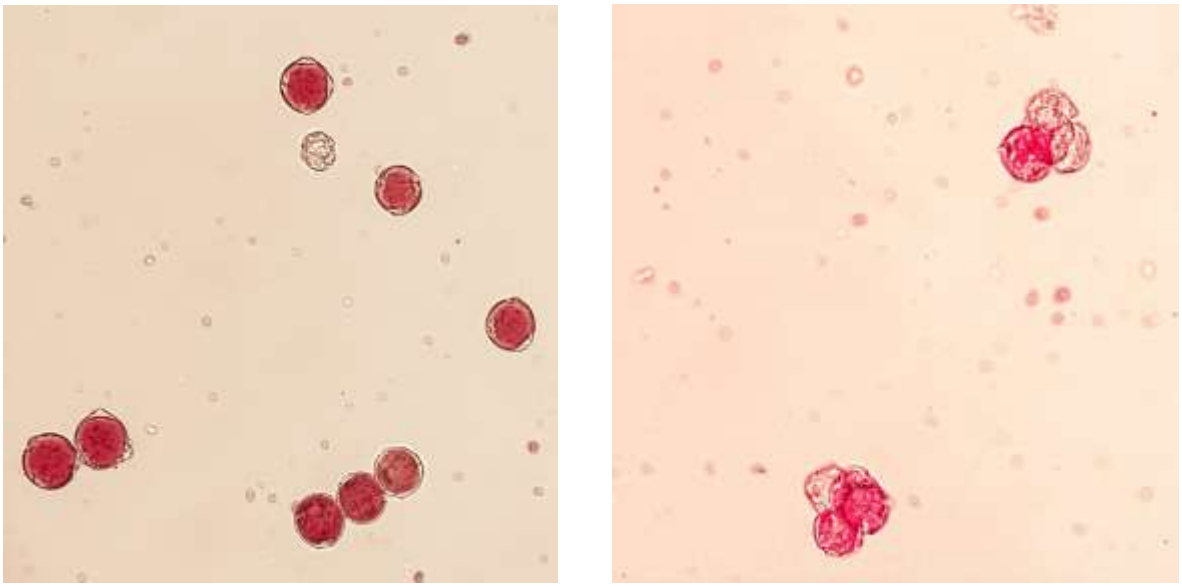

Figure 1. Potato pollen grains stained with acetocarmine, a) normal pollen grains of *S. tuberosum*, and b) potato pollen tetrad grains.

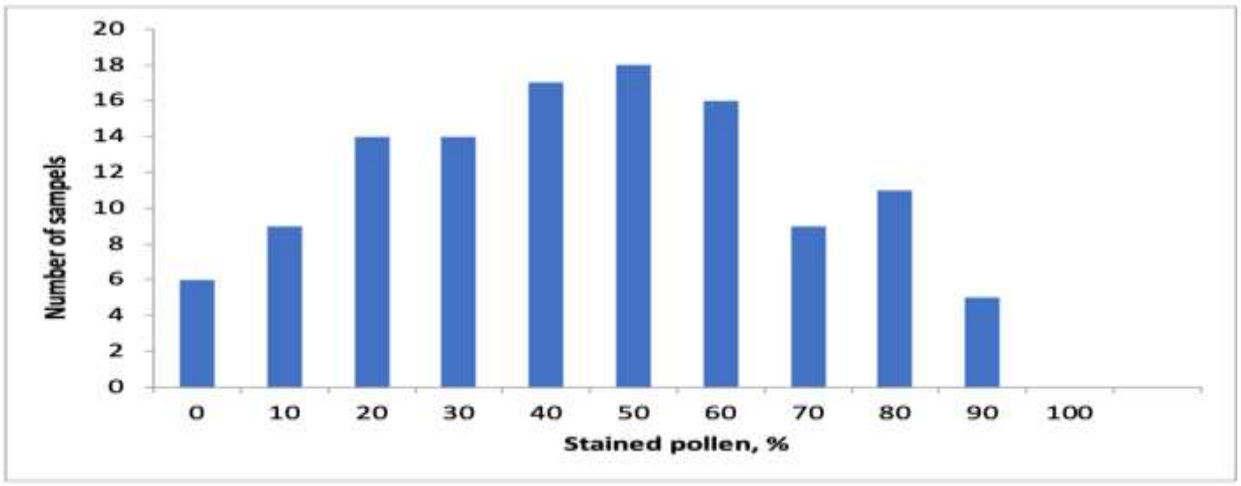

Figure 2. Distribution of potato samples by the percentage of stained pollen grains.

The percentage of stained pollen grains varied from 0% to 88.7% among the rest of the selection. In pollen samples of four cultivars (Nadezhda, Hibinskij dvuhurozhajnyj, Zol'skij, and Prinz) and two breeding samples, the colored pollen grains were completely absent. Two potato cultivars, Chugunka and Chaika, only displayed sporadic stained pollen grains (<1%) in the preparations. Another six samples also detected less than 10% of colored pollen grains. The maximum percentage of colored grains (more than 80%) revealed in the potato samples, viz., 3004-7 (88.7%), 3000-32 (86.5%), 21.32-1 (81.8%), 17.32-1 (80.3%), and cultivar Garant (86.8%). However, for most potato samples, the colored pollen grains ranged from 40% to 70% (Table 1, Figure 2).

In vitro germination test

All other potato samples (where no tetrad presence occurred) continued analysis through *in vitro* germination (Figure 3). The percentage of germination varied from 0% to 19.4%. Pollen grains germination did not occur

in 52 samples. For 20 samples, only sporadic pollen germination showed, with the percentage of germination at less than one. For the other 48 potato samples, the percentage of germinated pollen grains ranged from 1.3% to 19.4%. However, in seven samples (including four breeding lines 21.32-1, 18.5-17, 20.3-6, and 20.33-8, and three cultivars, Edison, Queen Anne, and Garant), the pollen percent germination scored more than 10%. In all potato samples, the distribution of germination frequency appears in Figure 4.

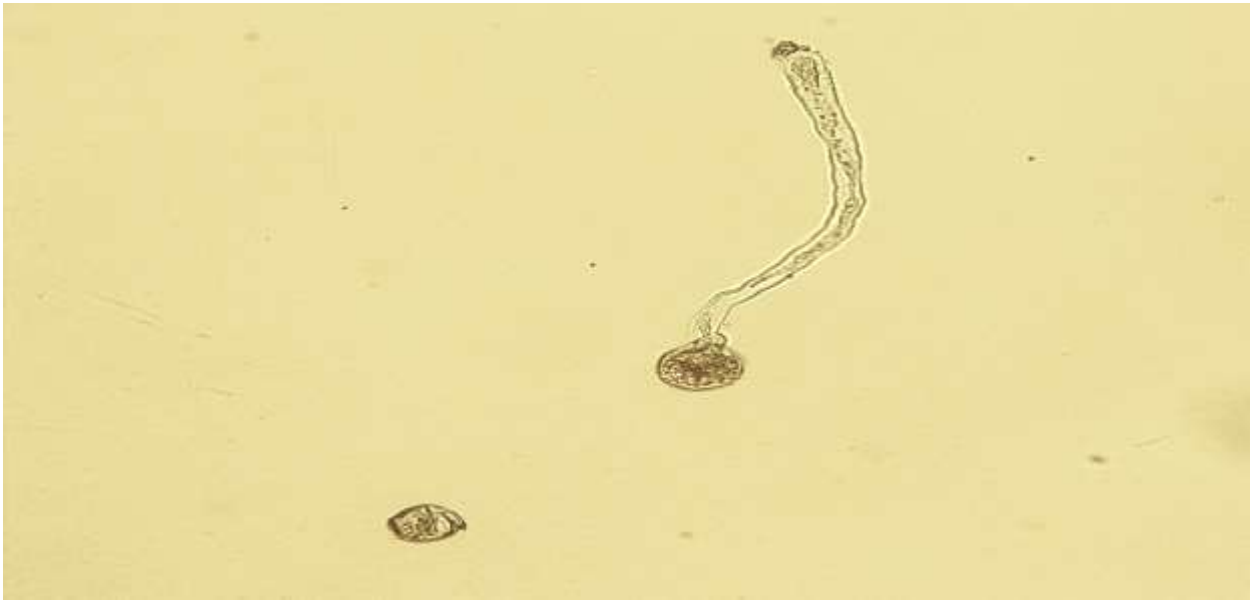

Figure 3. Germinated potato pollen grains.

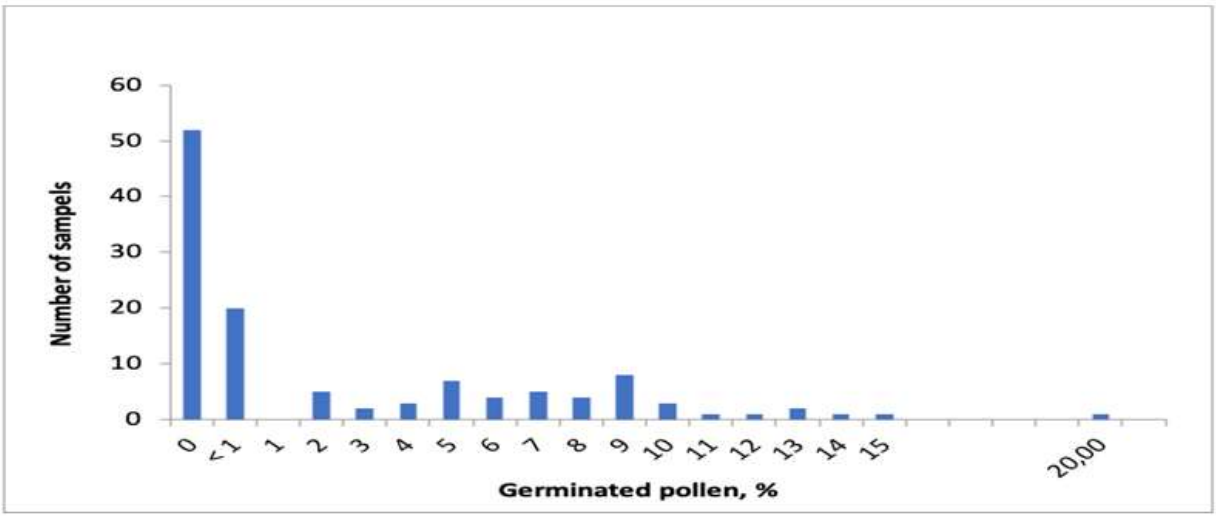

Figure 4. Distribution of potato samples by the percentage of germinated pollens.

Comparison of pollen fertility through acetocarmine staining and *in vitro* germination

The results obtained for the pollen fertility assessment of potato samples through two different methods differed significantly. Among 120 potato samples (which did not have tetrads), only 5% can be considered completely sterile based on the results obtained through acetocarmine staining. According to *in vitro* germination test, the number of sterile potato samples among these 120 samples scored 43.3%. For another 16.7% of samples, the germination percentage showed less than 1%. In all other cases, the pollen grains germination percentage ranged from 0% to 47.3% of the number of colored grains. However, only two potato samples (cultivars Naiada and Tarasov) resulted with more than 30% germination percentage of the stained pollen grains (Table 1).

Constructing a scattered plot ensued to visualize the results of pollen analysis by two different methods. As the graph shows (Figure 5), even the potato samples with a large percentage of stained pollen grains also have non-germinated pollens. Only the samples with a staining percentage of above 75% germinated well. At the same time, sample 17.32-1, which has 80.3% stained pollen grains, did not show the germination of a single pollen grain. The seven other potato samples (having 54% colored pollen grains) had a germination percentage higher than 10%.

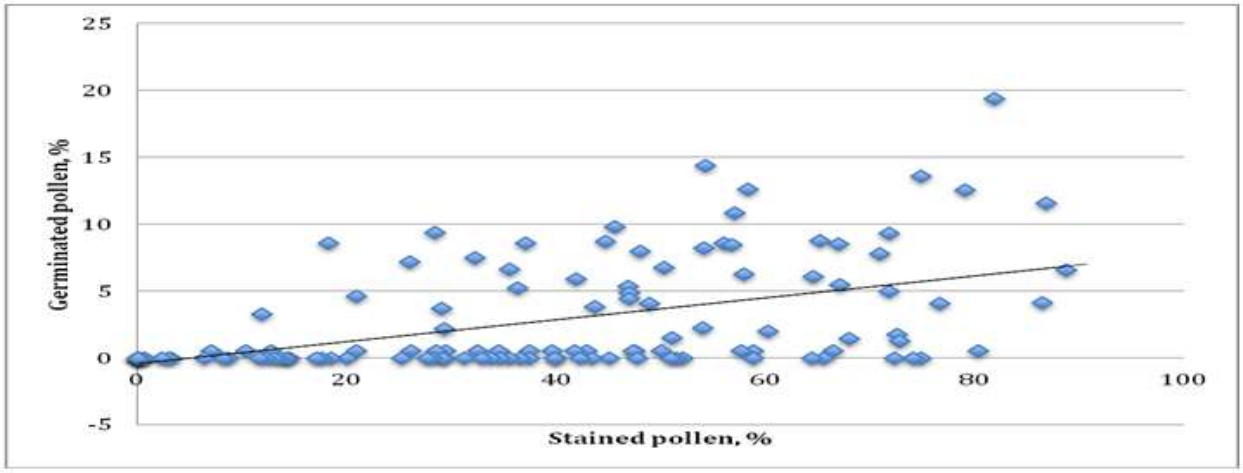

Figure 5. Scattering chart for stained and germinated pollens.

All potato samples with percent germination of more than 5% also had a percent colored pollen grains of more than 18% (Figure 5). The only sample for which a germination percentage gave more than 15% (19.4%) also had one of the highest pollen stainability indicators (81.8%). All other samples that did not have stained pollen grains confirmed no detection of germinating pollens. The trend line on the chart showed a positive correlation between the percentages of colored and germinating pollen grains.

Weather conditions

Since pollen fertility depends on weather conditions, the weather data collected during the crop season of 2022 got compared with the average long-term past data for the period from 2001-2019. The graph shows that in 2022 (Figure 6), from the second day of May until the end of the period, the rate of air temperature scored above the long-term average. During the pollen collection period from 26 July to 10 August, the average air

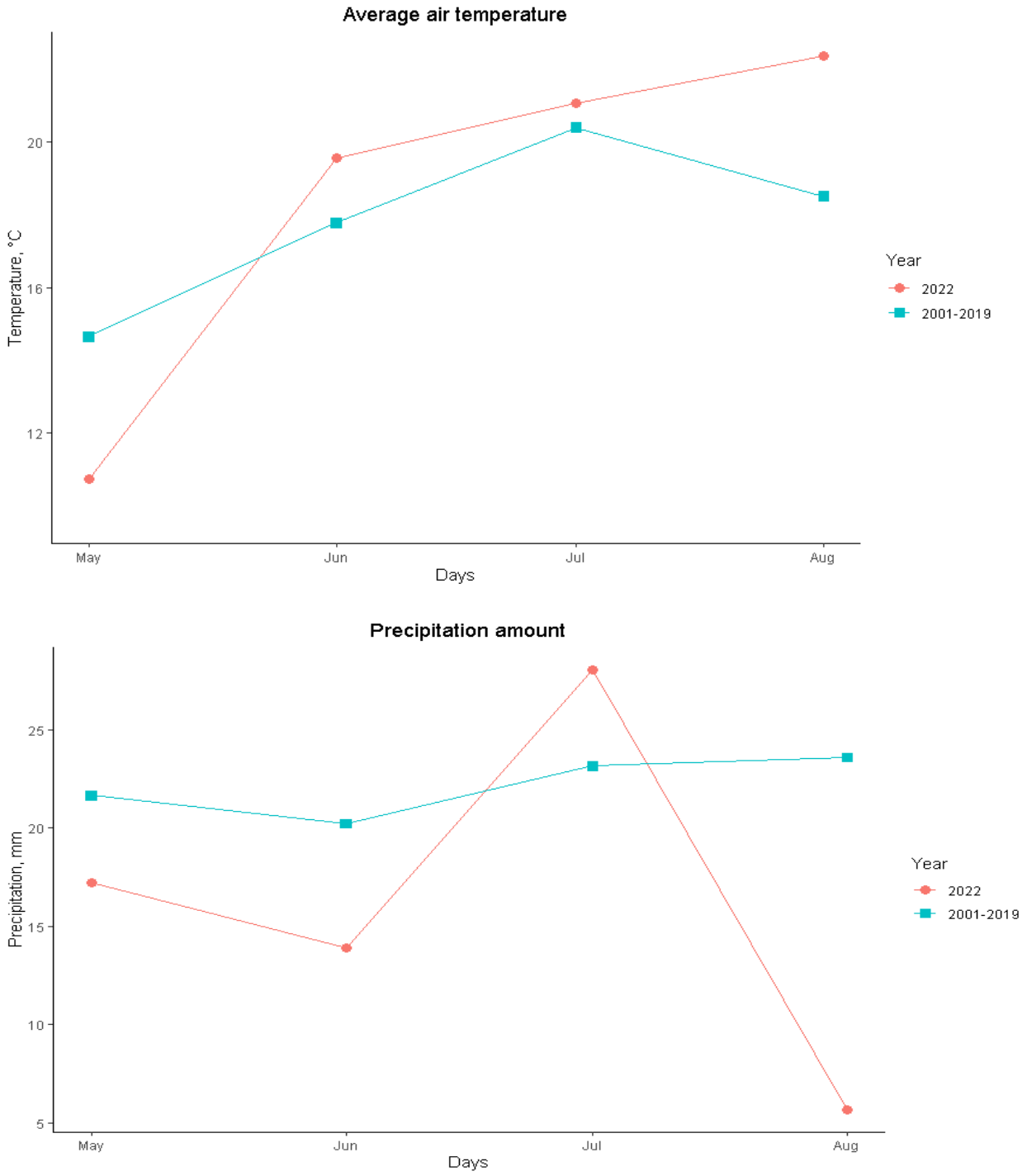

Figure 6. Temperature and precipitation graphs.

temperature reached 23.49 °C. During the experimental period of 2022, the study noted very heavy precipitation (55.9 mm) on the first day of July. In the following days, however, precipitation decreased sharply and, on average, amounted to 9.04 mm for five days. During the flower collection period from 26 July to 10 August, the precipitation measured 18 mm, which was less than the average for the period from 2001–2019. Thus, the year 2022 was drier and hotter compared with the average of the past 19 years.

DISCUSSION

According to the acetocarmine staining in the studied potato collection, the total percentage of sterile samples rated 18.3% (20 samples recorded with tetrad sterility and six samples with unstained pollen grains). However, the *in vitro* germination test showed the sterile potato samples at 50.7% (20 samples with tetrad sterility and 52 samples with non-germinating pollen grains).

According to the literature data in cultivated potatoes, three (T, W, and D) out of six types of cytoplasm link with the male sterility trait (Hosaka and Sanetomo, 2012), with the T-, W-, and D-types of cytoplasm as the most common. Thus, in the study of Japanese potato collection and European cultivars, the share of potato samples with these types of cytoplasm was 93.7% and 99.0%, respectively (Sanetomo and Gebhardt, 2015), and 99.5% among the potato cultivars bred in Russia and adjacent countries (Gavrilenko *et al*., 2019).

Thus, one can assume that in the study's collection also, more than 90% of the samples have T-, W-, and D-types of cytoplasm. However, the presence of germinating pollen grains came out for 49.3% of the samples, and for 35.2% of the samples, the percentage of germinating pollen grains scored more than 1%. These observations revealed compatibility with other researchers' data who also reported the sterile types of cytoplasm, also observing fertile pollens, which can be explained by nuclear-cytoplasmic interactions (Sanetomo *et al*., 2011, Gavrilenko *et al*., 2019).

In all 142 potato accessions, the presence of tetrads characterized 15.5% of the samples. The frequency of occurrence of potato samples with tetrad sterility was approximately the same among the analyzed potato cultivars and breeding lines (15.3% and 15.8%, respectively). One believes that this type of sterility links with the W-type of cytoplasm, originating from the species *S. stoloniferum,* then used as a donor of resistance to potato Y-virus (Lössl *et al*., 2000; Song and Schwarzfischer, 2008; Sanetomo and Gebhardt, 2015). Such type of cytoplasm was also previously found among European cultivars (12.2%), cultivars bred in Russia and adjacent countries (8.7%), and Japanese potato collection (2.4%) (Sanetomo and Gebhardt, 2015; Gavrilenko *et al*., 2019; Hosaka and Sanetomo, 2012). Thus, in the analyzed potato accessions, this type of cytoplasm occurs quite often, probably because the institute's collection supports samples with a complex of economically useful traits, including resistance to the Y-virus.

A known fact states that functional pollen sterility is typical for samples with the D-type of cytoplasm obtained from *S. demissum*. In this case, plants produce morphologically normal, well-stainable, but non-functional pollen grains (Dionne, 1961; Hosaka and Sanetomo, 2012). This possibly explains the fact that the results of the fertility assessment obtained by the two methods differ significantly, and 46 potato samples (32.4%) had stained pollen grains but did not germinate. Noteworthy to mention that according to Gavrilenko *et al*. (2019), 50.8% of bred cultivars in Russia and adjacent countries had the D-type of cytoplasm.

At the same time, it cannot be excluded that part of the variation in the results obtained by different methods is due to the influence of external factors that could affect the staining and germination of pollens in many ways. The analysis of weather conditions showed that during almost the entire growing season of 2022, the weather was hotter and drier compared with past long-term data, which could lead to a decrease in the ability of pollen to germinate. The most dependable way to assess the viability of pollen is to directly test the pollen's ability to pollinate and further seed formation (Thomson *et al*., 1994, Rodriguez-Riano and Dafni, 2000). However, this method proves labor-intensive, which limits its use on large scale. It should be noted that the formation of seeds clearly shows the fertility of pollen, however, their absence cannot work as an indicator of pollen sterility (Dafni and Firmage, 2000).

Currently, a considerable number of methods for assessing pollen viability *in vitro* exist; but have limitations and, therefore, a recommendation that several tests need simultaneous use to reflect multiple components of pollen performance (Thomson *et al*., 1994, Rodriguez-Riano and Dafni, 2000). The choice of the method also depends on the object of the study (Hanna and Towill, 1995). The comparative data on the assessment of potato pollen fertility using various methods on representative samples revealed extremely limited. Therefore, the results obtained proved beneficial for developing an effective protocol for assessing potato pollen fertility for further research on male pollen fertility problems and use in future breeding. The latest study made it possible to identify potato samples with a high percentage of stained pollen grains with a fairly high

percentage of germination. These potato samples can also serve as pollinators in future breeding programs.

CONCLUSIONS

The latest study presented the pollen fertility of 142 accessions of potato (*Solanum tuberosum* L.) assessed by two different methods, i.e., acetocarmine staining and *in vitro* germination. The results of the comparative estimation of potato pollen fertility obtained by the two methods differed significantly both in the number of identified fertile samples and in the assessment of the percentage of fertile pollen grains in the sample. These findings demonstrate the importance of the simultaneous application of several approaches based on different criteria rather than relying on a single method to score potato accessions reliably for the pollen fertility trait. Nonetheless, acetocarmine staining is a prompt, inexpensive, and efficient approach for high throughput screening applications and, hence, may be applied as preliminary evaluation for potato pollen fertility.

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REFERENCES

Dafni A, Firmage D (2000). Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Syst. Evol.* 222: 113-132.

Azka NA, Taryono, Wulandari RA (2021). Assessment of tea plant (*Camellia sinensis* L.) accessions for pollen sources in natural crossing by using microsatellites. *SABRAO J. Breed. Genet.* 53(4): 673-684. https://doi.org/10.54910/sabrao2021.53.4.10.

Dionne LA (1961). Cytoplasmic sterility in derivatives of *Solanum demissum*. *Am Potato J*. 38: 117-120.

Ellison W (1936). Meiosis and fertility in certain British varieties of the cultivated potato (*Solanum tuberosum* L.). *Genetica* 18: 217-254.

Fineman Z (1947). Elimination and retention of pollen sterility in potato improvement. *J. Agric. Res.* 75: 135-146.

Fukada Y (1925). Cytological studies on the development of the pollen grain in different races of *Solanum tuberosum* L. with special reference to sterility. *Bot.Mag.* 41: 459-474.

Gavrilenko TA, Klimenko NS, Alpatieva NV, Kostina LI, Lebedeva VA, Evdokimova ZZ, Apalikova OV, Novikova LY, Antonova Y (2019). Cytoplasmic genetic diversity of potato varieties bred in Russia and FSU countries. *Vavilovskii Zhurnal Genetiki i Selektsii - Vavilov J. Genet. Breed*. 23(6): 753-764.

Hanna WW, Towill LE (1995). Long-term pollen storage. *Plant Breed. Rev*. 13: 179-207.

Horčinová-Sedláčková V, Grygorieva O, Fatrcová Šramková K, Shelepova O, Goncharovska I, Mňahončáková E (2021). The chemical composition of pollen, staminate catkins, and honey of *Castanea sativa* Mill. *Potravinarstvo Slovak J. Food Sci*. 15(2021): 433-444.

Hosaka K, Sanetomo R (2012). Development of a rapid identification method for potato cytoplasm and its use for evaluating Japanese collections. *Theor. Appl. Genet*. 125(6): 1237-1251.

Iwanaga M, Ortiz R, Cipar MS, Peloquin SJ (1991). A restorer gene for genetic-cytoplasmic male sterility in cultivated potatoes. *Am. Potato J*. 68(1): 19-28.

Jansky SH, Charkowski AO, Douches DS, Gusmini G, Richael C, Bethke PC, Spooner DM, Novy RG, De Jong H, De Jong WS, Bamberg JB, Thompson AL, Bizimungu B, Holm DG, Brown CR, Haynes KG, Sathuvalli VR, Veilleux RE, Miller JC Jr, Bradeen JM, Jiang J (2016). Reinventing potato as a diploid inbred line. *Crop Sci.* 56(4): 1412-1422.

Kumar S, Prakash P, Kumar S, Srivastava K (2015). Role of pollen starch and soluble sugar content on fruit set in tomato under heat stress. *SABRAO J. Breed. Genet*. 47(4): 406-412.

Lindhout P, Meijer D, Schotte T, Hutten RCB, Visser RGF, Eck HJ (2011). Towards F_1 hybrid seed potato breeding. *Potato Res*. 54(4): 301-312.

Lössl A, Götz M, Braun A, Wenzel G (2000). Molecular markers for cytoplasm in potato: Male sterility and contribution of different plastid mitochondrial configurations to starch production. *Euphytica* 116: 221-230.

Ordoñez B (2014). Brochure: Pollen Viability Assessment. International Potato Center (CIP), Lima, Peru. pp. 8.

Ortiz R, Iwanaga M, Peloquin SJ (1993). Male sterility and 2n pollen in 4x progenies derived from 4x × 2x and 4x × 4x crosses in potatoes. *Potato Res*. 36(3): 227-236.

Rodriguez-Riano T, Dafni A (2000). A new procedure to assess pollen viability. *Sex. Plant Rep.* 12: 242-244.

Salaman R (1910). Male sterility in potatoes, a dominant Mendelian character; with remarks on the shape of the pollen in wild and domestic varieties. *Bot.J. Linnean Soc.* 39: 301-312.

Sanetomo R, Gebhardt C (2015). Cytoplasmic genome types of European potatoes and their effects on complex agronomic traits. *BMC Plant Biol*. 15(1): 162.

Sanetomo R, Ono S, Hosaka K (2011). Characterization of crossability in the crosses between *Solanum demissum* and *S. tuberosum*, and the F_1 and BC_1 progenies. *Am. J. Potato Res.* 88: 500-510.

Song Y-S, Schwarzfischer A (2008). Development of STS markers for selection of extreme resistance (*Rysto*) to PVY and maternal pedigree analysis of extremely resistant cultivars. *Am. J. Potato Res*. 85(2): 159-170.

Stanley RG, Linskens HF (1974). Pollen: Biology, biochemistry, and management. Springer, New York.

Stout A, Clark CF (1924). Sterilities of wild and cultivated potatoes with reference to breeding from seed. US Department of Agriculture (USDA), USA, *Bull. No.* 1195.

Thomson JD, Rigney LP, Karoly KM, Thomson BA (1994). Pollen viability, vigor, and competitive ability in *Erythronium grandiflorum* (Liliaceae). *Am. J. Bot*. 81: 1257-1266.

Trognitz BR (1991). Comparison of different pollen viability assays to evaluate pollen fertility of potato dihaploids. *Euphytica* 56: 143-148.

Visser T (1955). Germination and storage of pollen. *Meded Landbouwshogesch Wageningen/Nederland* 55: 1-68.

Zubkova T, Motyleva S, Vinogradov D, Gulidova V, Dubrovina O (2022). Organic fertilizer and natural zeolite effects on morphometric traits of *Brassica napus* L. pollen grains. *SABRAO J. Breed. Genet*. 54(2): 397- 406.