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Pollen viability and *in vitro* germination of selected Central European species from genus *Rosa* analysed with different methods

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Abstract: The main aim of the study was to determine which of the viability and germination capacity of pollen grains estimation methods are optimal and to test if the studied sections and species within the *Rosa* genus are differentiated in pollen viability and germination capacity. We analysed and compared pollen grains of 14 wild *Rosa* species using 5 viability tests and 13 various liquid and agar media. Viability of pollen grains in the majority of the examined species was greater than their germination capacity. The most viable pollen grains were found in *R. gallica* (section *Gallicanae*), where average of 5 viability tests is 90.69%, and in *R. pendulina* (section *Rosa*) – 86.85%. Species from section *Caninae* have a lower level of viability (from 60.59% in *R. rubiginosa* to 31.23% in *R. inodora*). Pollen of species from sections *Gallicanae* (*R. gallica*; to 63.4%) and *Rosa* (*R. pendulina*; to 47.21%) germinated much better than the pollen of the majority of species from section *Caninae* (with the exception of *R. zalana*; to 47.22%). The examined pollen grains germinated most numerously on agar medium with 1.5% agar+15% sucrose+50 ppm boric acid and liquid medium with 15% sucrose+50 ppm boric acid. Following deep-freeze storage (–25°C) for six months, the pollen grains of nine selected species (with the exception of *R. rubiginosa*) showed viability higher or similar to that before storage.

Additional key words: Rosaceae, TTC, FCR, Müntzing test

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Introduction

In Europe there are 47 *Rosa* species, belonging to 5 section (Klaštersky 1968). Henker (2000) reports about occurring 33 species of genus *Rosa* in Central Europe. Most of the European roses including *Rosa* in Poland, belong to the section *Caninae* (Gustafsson 1944; Klaštersky 1968; Zieliński 1985; Henker 2000).

The subject of these studies were 14 *Rosa* species, naturally occuring in Central Europe, whose pollen was examined for viability and germination (Table 1). These roses belongs to three sections (Zieliński 1987; Popek 1996; Henker 2000).

The studies on pollen viability and germination capacity are important in the case of difficult group of plants of hybrid nature and not determinated taxonomic position, to which belongs genus Rosa and their representative of *Caninae* section. This section is characterized by polyploidy (usually pentaploidy) and a unique meiosis (Zieliński 1985; Werlemark 2000). Jicinska et al. (1976) reported that the male meiosis often is unsuccessful and that pollen quality within

this section is very poor. According to a study conducted by Kroon and Zeilinga (1974) on section *Caninae*, the percentage of empty pollen grains varies from 30 to 70%. Because hybrids have malformed pollen grains, the studies on pollen viability and germination capacity allow to determine the nature of hybrid of individual taxons using simple methods and also select the species of the highest pollen viability and germination capacity and therefore possibly confirm their status of "good species".

The results of such studies could also indicate the possibility of routine application of some examined roses for breeding in the case of improving varieties with resistance and fertility by using them as specimens of the parents in receipt of a stronger, fertile off springs (Khosh-Khui et al. 1976; Voyiatzi 1995; Günes et al. 2005; Ercisli 2007; Zlesak et al. 2007; Wang et al. 2009).

The literature concerning the studies on pollen viability of *Rosa* species is meager. The first researches in this field were made by Täckholm (1922), who studied germination of pollen. Mameli Calvino (1951), Wohlers et al. (1962), Peimbert et al. (1963), Wohlers and Morey (1963), Visser et al. (1977) and Pearson and Harney (1984) studied the pollen germination *in vitro* under different conditions and using cotton blue testing. They examined viability of pollen mostly in cultivars. Some of the studies also focused on the influence of storage duration on pollination. Kon alova (1975) studied the pollen germination *in vitro* of *R. hugonis*. Sykorova et al. (1976) compared the different results of estimation of pollen germination using TTC (triphenyltetrazolium chloride) test. The authors extended the range of studies of pollen viability and germinability of eight *Rosa* taxa based on the TTC test and few types of germination media with sucrose and 1.5% agar.

In recent investigations the pollen germination of *Rosa* has been estimated in liquid media with sucrose concentration of 10, 15 and 20% or agar of 1 and 1.5% (Ueda and Hirata 1989; Voyiatzi 1995; Günes et al. 2005). Günes et al. (2005) have also used different concentration of boric and gibberellic acid. Ueda and Hirata (1989) focused mainly on the studies of relationships between pollen germination and the concentration of boric acid and temperature and Voyiatzi (1995) described the effect of pH of the medium on pollen grains germination. The viability of pollen grains of *some Rosa* species and cultivars have been analysed by comparing the efficiency of using different tests of viability of pollen grains e.g. the cotton blue test (Werlemark 2000), TTC test (Günes et al. 2005; Ercisli 2007) and IKI test (Ercisli 2007). The most recent and common method in estimation of pollen viability is the FCR (fluorochromatic test procedure), often compared with other methods of pollen viability estimation as test for enzyme activity (TTC) or test of stainability of the vegetative cell contents (Heslop-Harrison and Heslop-Harrison 1970; Heslop-Harrison et al. 1984; Shivanna et al. 1991). The FCR method tests the integrity of plasmalemma of the vegetative cell and it expressed higher correlation to germination in comparison to histochemical methods (tests of stainability of cell contents, tests for enzyme activity).

In present studies the stainability test (Müntzing test) as well as test for enzyme activity (TTC: triphenylotetrazolinum chloride) have been used mainly for comparison with FCR procedure used here for this first time.

The FCR procedure is based on the fluorochromatic reaction of plasmalemma of the vegetative cell of the male gametophyte. Up until now this is the most accurate and proper estimation of potential viability and germination of pollen grains. The viability of male gametophyte is closely related to the state of plasmalemma. If this shows the normal permeability, the cell is likely to be viable. The basis of the FCR method is the phenomenon of fluorochromasia, described by Rotman and Papermaster (1966).

The use of the FCR in present studies (Heslop-Harrison et al. 1984; Shivanna et al. 1991; Rhee et al. 2003; Nepi et al. 2005) may provide an effective method for assessing pollen quality: pollen germination capacity and the estimation of their viability more precisely. Besides the tests mentioned, the viability pollen of examined *Rosa* species has been also estimated in non stained test and with "interference contrast".

In order to determine the best species, tests on the viability of pollen stored for 6 months in deep freeze was also carried out. Zlesak et al. (2007) and Wang et al. (2009) have provided similar studies after storage of pollen of some *Rosa* cultivars.

Besides of numerous studies focused on viability and germination of pollen grains of rose species, the knowledge on this theme it is not sufficient. he results which have been obtained hitherto, were concerned usually with cultivars of roses, (only a few *Rosa* species have been examined) and were made using one viability test, often TTC. The germination of pollen has been tested on one or few media.

Therefore the aim of these studies reported in this paper is the comparison of pollen quality of several *Rosa* species using numerous, various viability tests and *in vitro* germination on various test media, with various concentrations and contents (liquid or solid with addition of boric acid, sucrose, maltose, lactose) and an estimation of which of them could be suitable to assess pollen viability and germination. Fourteen wild rose species from the sections *Rosa, Caninae* and *Gallicanae* were used to test the various methods.

Materials and methods

Flower buds and flowers from the terminal shoots of 26 rose bushes were collected in May and June 2007 and 2008 from the Adam Mickiewicz Botanical Garden, University in Poznań, Dendrological Garden of Poznan University of Life Sciences and from the plant collections in Kórnik Arboretum (in Kórnik). The origin of these plants were wild localities in Poland, Czech Republic, Hungary, Germany and Austria. The inflorescences of plants were brought into the laboratory in bud and flowering state. Roses flowered over a short interval short, because of very high temperatures in those years, therefore the plant material have been stored in a refrigerator at 8°C soon after collecting within about one–two days. The rose shoots were moved to the laboratory and pollen was collected from fleshy dehiscing anthers, which were dusted from newly opened flowers. Usually anthers released pollen grains in about 3–8 hours during the day. Pollen grains from freshly dehisced anthers were spread on a slides from 3 separate flowers of each species. The rest of the anthers and pollen were stored in deep freeze within 3–6 months for further studies.

Up until now, as far as we know, the differences viability and germination of pollen between the *in vitro* and *in vivo* examination have not been examined for roses. Therefore in present studies we taking into account to minimalize the valid influence of outdoors factors like insufficient humidity, too high temperature, not enough sterile media, laboratory tools or assistance (not enough sterile conditions in germination tests).

Pollen viability

The viability of pollen was first estimated using non enzymatic and non staining tests in a preliminary (survey). The viability of pollen was preliminary tested on fresh pollen, dusted on 15% sucrose liquid medium on slides. Mature and correctly formed pollen grains with morphological features typical for roses were tested only. The morphological features taking into consideration were according to descriptions by Erdtman (1952), Reitsma (1966), Eide (1981), Hebda and Chinnappa (1990), Moore et al. (1991), Wrońska-Pilarek and Jagodziński (2009) and Wrońska-Pilarek (2010).

They were not collapsed, contained cytoplasm and exhibited the size parameters comparable to data in the literature. Non viable grains differ from viable grains by abnormal shape, size and exine colour (Stanley and Linskens 1974).

In the aim of more detailed preliminary assessment of non stained pollen, grains were observed under the microscope in "interference contrast". The next test, which evaluates the viability of pollen and their ability to fertile was procedure of Müntzing, described by Löve and Löve (1975). This method distinguishes filled and empty sterile pollen and indicates the pollen fertility better than separating the viable and non-viable pollen (Löve and Löve 1975).

The potential pollen viability test was carried out on fresh material using enzymatic methods (TTC-tetrazolinum method) following the procedure of Oberle and Watson (1953) and fluorescence method (FCR) recommended by Heslop-Harrison and Heslop-Harrison (1970). In current studies the method is used in accordance with Shivanna and Heslop-Harrison (1981) and Bernhardt and Edens (2004). In the fluorescence method of Bernhardt and Edens (2004) fluorescent diacetate (FDA) was dissolved in acetone (20 mg FDA in 10 ml acetone). This marker called "stock solution" was made up in at least in one day advance and it was stored in a freezer until needed, but no longer than 1 month. Immediately before FCR testing, the 10% sucrose solution was made in distilled water. The "stock solution" was added drop by drop to this sucrose solution until it turned milky. Such milky solution was placed as a drop on the slide and the pollen was dusted on. The pollen grains were bathed in this solution for ten minutes on the cover – slip was placing. The examination was taken under the fluorescence lamp in a microscope. The potential viability was estimated on the intensity of shining. The interior of healthy grain turns yellow ("the head"), whereas the dead grains stay dark.

The preliminary assessment of pollen were taken into consideration also in testing of stored pollen in "deep freeze" (Müntzing test, non stained test) after 6 months.

For each test from 500 to 1000 pollen grains per species were used. All together 40,000 pollen grains were tested. Because of all of the *Rosa* species were blooming almost at the same time, the estimation of viability was also possible using the electronic photo-images. This electronic colour photos documentation is deposed in the Department of Forestry Natural Foundations, Poznan University of Life Sciences.

Pollen germination

Pollen germination capability was examined for 13 wild *Rosa* species using 13 various liquid and agar media. Each species was represented by 500 pollen grains to a total 84,500 pollen grains.

In vitro pollen germination was induced in sterile Petri dishes under stable humidity according to standard methods (Stanley and Linskens 1974) using various combination of liquid media containing boric acid or without in concentration of 10, 15 and 20% of sucrose. Germination on permanent media was tested using 1.5% agar with combination of sucrose (10, 15%) and boric acid or without. Three agar me-

dia contained 12% lactose or 12% maltose with or without boric acid have been used.

In all cases the concentration 50 ppm of boric acid was applied. The fresh pollen was dusted directly onto slides with the media and put in the sterile Petri dishes to stimulate germination atg 25°C. The first observations were made after 24 hours for agar media and after 2–4 hours for liquid ones and repeated every day, all together 10 days. The criterion for the germination was a well formed pollen tube which was longer than the pollen diameter (Shivanna and Heslop-Harrison 1981). Pollen grains were tested generally on 6 liquid media and on 7 stable ones. Some of the slides were mounted with Müntzing reagent and were tested in FCR procedure as possible. The estimation of pollen capability to germinate was performed on 95 different media for all species.

The percentage of germination ability and viability of pollen was calculated also using electronic images taken from LM microscope usually in magnification of ×1000.

Results

All studied pollen grains are mostly 3-zonocolporate, rarely pollen with 4 colpi and pori (4-zonocolporate) occur (*R. canina, R. micrantha* and *R. jundzillii*).

Section: *Rosa*

Rosa pendulina L.

1. Pollen viability

The quality of non-stained pollen was very high and its viability was estimated using survey methods as approximately 90% (Table 1). The most of pollen was typical for *Rosa* type, well formed, and only a few grains were as malformed. The pollen viability tests gave similar, very high results (Müntzing test – 92.78% and TTC test – 90.05%). The fluorochromatic reaction (FCR) gave values of 89.15%.

2. Pollen germination

Germination of pollen grains of *R. pendulina* varies according to method (Table 2). The optimal medium for pollen of this species is liquid medium – 15% sucrose, with a value of 47.21% approximately. In FCR reaction 45.45% of pollen were alive and germinated, whereas using "interference contrast" the pollen germination was 46.8%. The addition of boric acid for 15% sucrose liquid medium (50 ppm) limited mould development, however it decreased the germination to 39.9%.

The pollen germination is fast in liquid media like such as 10%, 20% and 20% sucrose with 50 ppm of boric acid but pollen tubes often burst (because of the low solute concentration compared to cell sap). On agar media the pollen germinated more slowly and were longer alive. In agar media with 10% sucrose +50 ppm of boric acid and in agar with 15% sucrose pollen have geminated similar as in liquid media and performed 39.43 and 41% respectively. Pollen grains dusted on agar with 10% sucrose, have exhibited high viability, approximately 69.12% .

Poor germination of *R. pendulina* pollen was observed on agar media with lactose and maltose, although the pollen seemed to be alive. Pollen tubes were approximately 100 μm long in liquid media and approximately 200 μm long on agar media.

Section: *Caninae*

Rosa agrestis Savi, *R. canina* L., *R. dumalis* Bechst., *R. inodora* Fr., *R. jundzillii* Besser, *R. micrantha* Borrer ex Sm., *R. mollis* Sm., *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm., *R. villosa* L., *R. zalana* Wiesb.

The studies on viability and germination on some species of the section *Caninae* have faced with difficulties concerning with a great number of malformed, collapsed pollen grains usually with empty contents (without cytoplasm). Taking into account *R. villosa*, only about 30 living pollen per 320 malformed were observed under the microscope examinations.

The similar condition of pollen has been found for *R. sherardii*. Here, in the aim for viability estimation based on stain ability reaction, almost 1300 pollen should have been tested. The estimation of pollen germination level for these species could be difficult, because some of pollen grains not only germinate later than the others but also the only one trace of germination of these malformed grains are the remains of swollen and bursted pollen tubes just near the porus. During the studies we could not observed the process of germination in most of the living pollen in the some time, so the several repetitions of observation were necessary to calculate the capability of germination. The observation has been performed almost every day (6–7 times) and even 10 days after dusting the pollen on the media.

1. Pollen viability

The examined species of the section *Caninae* showed remarkable differences of pollen viability within particular tests (e.g. Müntzing test from 15.1 to 50.01%; TTC in 29.43–54.56%; FCR in 35.22–87.58%; Table 1).

The most viable pollen seems to have *R. zalana*. In viability test, the pollen have indexes of approx. 50.01% (Müntzing test), 53.28% (TTC) and 66.35% (FCR). Then *R. rubiginosa* with indexes relatively 46.06%, 54.56% and 87.58% followed by *R. dumalis* with data of 48.2%, 32.56% and 62.24% relatively. Within the remaining species the indexes of pollen viability presented variable level. The differences cover the range from 21.09% (TTC test) to 43.66% (prior viability test of non-stained pollen). In the other tests

Table 1. Viability of pollen of studied *Rosa* species. Bold – viability of pollen of some species after 6 month storage in deep freeze, – insufficient quantity of material for testing

No.	Section	Species	Müntzing test [%]	TTC test [%]	FCR test [%]	Interference contrast [%]	Viability test of non-stained pollen [%]
1	*Rosa*	*R. pendulina*	92.78	90.05	89.15	88.72	92.75
2	*Caninae*	*R. agrestis*	30.86	29.43	65.75	64.0	36.81
3	*Caninae*	*R. canina*	34.27	38.34	65.60	30.89	29.80
			47.86				
4	*Caninae*	*R. dumalis*	48.26	32.56	62.24	49.56	44.00
5	*Caninae*	*R. inodora*	25.35	–	37.10	–	–
6	*Caninae*	*R. jundzillii*	15.51	50.52	49.05	17.52	54.16
			17.36				
7	*Caninae*	*R. micrantha*	33.52	33.19	56.33	46.49	31.62
			49.46				
8	*Caninae*	*R. mollis*	43.15	49.90	35.22	37.41	51.09
			53.44				
9	*Caninae*	*R. rubiginosa*	46.06	54.56	87.58	70.92	43.81
			15.5				
10	*Caninae*	*R. sherardii*	38.36	38.26	37.36	28.48	31.18
11	*Caninae*	*R. tomentosa*	24.01	36.18	53.49	49.54	49.78
			18.24				
12	*Caninae*	*R. villosa*	41.09	33.90	46.81	38.35	10.50
			31.99				
13	*Caninae*	*R. zalana*	50.01	53.28	66.35	56.63	65.00
			76.1				
14	*Gallicanae*	*R. gallica*	92.24	92.67	85.43	74.64	89.25
			90.4				

Table 2. The effects of different liquid and agar media action on capability for pollen germination in studied *Rosa* species (expressed in percentage)

Species	Medium type												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	liquid media							agar media					
R. pendulina	+/–	47.21	–	<10	39.93	+/–	39.43	35.51	25.51	41.25	+/–	<2	–/ live
R. agrestis	6.57	22.2	10.25	–/ live	14.05	5.98	–	20.4	–	10.26	–/ live	–/ live	–/ live
R. canina	7.64	=1	5.73	<1	<1	12.81	14.92	7.56	3.61	2.65	–/ live	–	13.82
R. dumalis	+/–	+/–	–	–	3.35	–	–	20.4	8.89	9.37	–/ live	–/ live	–/ live
R. jundzillii	7.48	2.63	5.0	3.61	5.2	14.72	15.26	2.5	24.8	17.15	–	1.04	–
R. micrantha	–/ live	–	15.21	–/ live	14.9	–	–	–/ live	–/ live	–/ live	–/ live	–/ live	–/ live
R. mollis	–/ live	–/ live	–/ live	–/ live	28.09	–/ live	–	24.21	–/ live	–/ live	–/ live	–/ live	–
R. rubiginosa	–	20.45	+/–	–	–	–/ live	–/ live	2.17	–/ live	–/ live	–/ live	–/ live	–/ live
R. sherardii	27.77	–	–	–	–	9.27	–	16.65	<10	1	–	–	–/ live
R. tomentosa	6.89	–	14.44	–/ live	–/ live	2.89	7.27	12.03	–/ live	1	1	3.33	–
R. villosa	3.35	3.4	5.02	< 2	–	6.76	< 2	–	< 2	–	–	–	–
R. zalana	7.48	–	47.22	–	26.07	23.07	42.27	42.56	+/–	+/–	–/ live	–/ live	15.95
R. gallica	60.23	63.4	59.87	25	35.56	37.48	45.16	10.12	9.94	15.12	25.2	35.92	43.08

Medium type: liquid media: 1 – 10% sucrose; 2 – 15% sucrose; 3 – 20% sucrose; 4 – 10% sucrose+50 ppm boric acid; 5 – 15% sucrose+50 ppm boric acid; 6 – 20% sucrose+50 ppm boric acid. Agar media: 7 – 1.5% agar+10% sucrose+50 ppm boric acid; 8 – 1.5% agar+15% sucrose+50 ppm boric acid; 9 – 1.5% agar+10% sucrose; 10 – 1.5% agar+15% sucrose; 11 – 1.5% agar+12%; lactose+50 ppm boric acid; 12 – 1.5% agar+12% lactose; 13 – 1.5% agar+12% maltose+50 ppm boric acid.
– not germinating pollen; –/live – pollen not germinating but living; +/– – pollen germinating, but pollen tubes dehiscent.

the differences showed approximately 30%. The interesting results have been obtained for *R. jundzilli*, which indeed produce huge quantities of pollen, mostly malformed and without cytoplasm, but distinguished by high enough the indexes of TTC and FCR tests (50.52 and 49.05% respectively).

Remarkable pollen viability showed *R. mollis*, with high indexes of Müntzing and TTC test (43.14 and 49.9% respectively) but low index of FCR test (35.22% only). The next species in the queue of pollen viability indexes is *R. sherardii*. The indexes obtained were as following: FCR – 37.36%; TTC – 38.26%, Müntzing test – 38.36%). The lowest index of pollen viability within the section *Caninae* presented *R. villosa* (TTC 33.9%; FCR 46.81%) and *R. inodora* (Müntzing test 25.35%; FCR 37.1%).

In present studies we used the Müntzing test also for the estimation of pollen viability after 6 months storage in deep freeze (–25°C; Table 1) in following species: *R. canina, R. jundzillii, R. micrantha, R. mollis, R. rubiginosa, R. tomentosa, R. villosa, R. zalana*. Most of the species (*R. canina, R. jundzillii, R. micrantha, R. mollis, R. zalana*) have showed even higher viability in 2–16% than before storage; *R. zalana* pollen were more viable than the others and reach even 26%. A little lower viability after storage has been detected for *R. tomentosa* and *R. villosa* pollen and much lower only in *R. rubiginosa* (approx. about 30%).

2. Pollen germination

Pollen grains of the section *Caninae* have germinated in variable level (Table 2). Pollen grains of some of the species have not germinated on the majority of the media, although in some media pollen of *R. dumalis, R. micrantha, R. mollis, R. rubiginosa* or *R. sherardii* seems to be alive even till 10 days after dusting especially for *R. mollis* and *R. sherardii* (using Müntzing stain to detect). The pollen grains of the following species: *R. agrestis, R. canina, R. jundzillii, R. zalana* have germinated in almost all media. Many of these grains developed long pollen tubes but in some of the species like *R. canina, R. dumalis, R. rubiginosa,* although the pollen have germinated very well, the pollen tubes have dehiscent soon after the beginning of germination.

Somewhat more pollen have germinated on liquid media than in agar ones. Almost for all examined species for this section, the optimum was the liquid medium with 15% sucrose+50 ppm boric acid, where the most numerous germinated pollen grains belonged to *R. mollis* and *R. zalana* (in 28.09 and 26% respectively). Optimal enough turned to be liquid medium with 10, 15 and 20% sucrose. The first one was the best for *R. sherardii* pollen (27.77%), the second for *R. agrestis* (22.2%) and *R. rubiginosa* (20.45%), and the last one for *R. zalana* (47.22%). A few pollen have germinated on liquid media with 10% sucrose+50 ppm boric acid. For liquid media the poor germination of pollen has been detected in *R. dumalis* and *R. mollis*, each germinated only on one among six media (15% sucrose+50 ppm boric acid). *R. dumalis* has germinated very poor in 3.35% whereas *R. mollis* on relatively high level (28.09%).

From among the agar media, the optimum was medium with 15% sucrose+50 ppm boric acid, where the pollen of *R. zalana* germinated the most numerous (42.56%), and numerous enough – *R. mollis* (24.21%) and *R. dumalis* (20.4%). On the rest combination of agar media, the pollen of singular species have been germinated only. It has been presented as follows: on agar medium with of 10% sucrose+50 ppm boric acid has obtained high result for *R. zalana* (42.56%); for *R. agrestis* and *R. dumalis* – agar medium with 15% sucrose (10.26% and 9.37%). Pollen of such species as *R. micrantha, R. villosa* and *R. rubiginosa* have showed very poor germination on the mentioned agar media. Pollen of *R. micrantha* have not germinated at all and the pollen of the two remain species have hardly germinated on one or two media and on very low level (2–3%).

The germination of pollen of the species examined has been also tested in three different agar media for the first time in this studies. We used agar media with 12% lactose+50 ppm boric acid, 12% lactose and with 12% and maltose+50 ppm boric acid here. The pollen of the most examined species have not germinated on these media, although pollen were alive (we were using Müntzing stain to detect). Pollen of some species (*R. jundzillii, R. tomentosa*) have germinated in very low level as following: 1.04 and 3.33%, somewhat better *R. canina* (13.82%) and *R. zalana* (15.95%).

The pollen tubes length of the species of section *Caninae* have been differentiated. On the liquid media the pollen tubes reached the length of approximately 60–350 μm; usually 120–160 μm. Their length were similar on agar media (up till 300 μm). In some of the media the pollen grains with developed two pollen tubes, have been observed (*R. zalana* on liquid media 20% sucrose+50 ppm boric acid).

Usually the pollen grains have developed the longest pollen tube on optimum germination media and of course their index of germination was very high. For *R. zalana,* agar media with 10% sucrose turned out to be optimum to germinate numerously and develop pollen tube up till 300 μm. We have found it similar for *R. mollis* pollen which develop tube up 214 μm; *R. villosa* up to 240 μm or *R. agrestis* up to 350 μm on the agar media mentioned above. However it has been also observed that pollen of *R. jundzillii* have developed very long tube (up till do 210 μm) on the liquid medium with 15% sucrose, despite of very poor germination (2.63%).

Section: *Gallicanae*

***Rosa gallica* L.**

1. Pollen viability

All tested pollen grains of this species were developed and filled up with cytoplasm contents. The malformed and collapsed pollen have almost not been noticed. The results of viability tests were on equally very high level. The highest values have been obtained in TTC test (92.67%) then Müntzing test (92.24%) and at least the prior tests of estimation of quality and viability of non stained grains (89.25%) and the values of "interference contrast" (74.64%; Table 1). Only somewhat lower values of pollen viability the FCR test has shown (85.43%).

2. Pollen germination

The germination of pollen grains of *R. gallica* is also on very high level. It is versatile (universal) and the only one species from the examined ones, whose grains have germinated on all media (Table 2). The optimal were liquid media with 10, 15 and 20% sucrose. Pollen have germinated on equal level from 59.8 to 63.4% (Table 2). The best results we obtained on liquid medium with 15% sucrose: 63.4% with viability approximately 90%. Somewhat less pollen have germinated also on liquid media with 10 and 20% sucrose (60.23, 59.83% respectively). The viability has been also high and reached 85–90%. Pollen germination has turned out to be lower on agar media. The optimum medium was agar with 10%sucrose+50 ppm boric acid, where grains have germinated in 45.16% with viability approximately 92%.

Because of the universality of germination of *R. gallica* pollen, some trials on agar media with 12% lactose and 12% maltose have been made. The values which we obtained: 35.92% and 43.08% respectively, have been the highest values of germination within the examined species taking into consideration also very high viability: 94–98%.

The longest pollen tubes have been developed on liquid media, with 20% sucrose especially creating tubes of 68 to 200 μm approximately. The similar scope of length presented pollen tubes on agar media; the longest were 210 μm on agar with 10% sucrose; on agar media with lactose the pollen tubes were shorter – the range 66–105 μm and on agar with maltose much shorter than previous one.

The additional estimation of pollen viability of *R. gallica* has been detected after 6 months storage in deep freeze (–25°C) using Müntzing test. The pollen viability has shown very high value of the range 86.3–94.7% and with mean value of 90.4%. The results of the viability test before and after storage are very similar.

Discussion

There is a linear relation between pollen viability and germination capability in many fruit species (Grigs et al. 1971). The high viability the highest germination capability. Though in present studies such linear relation was demonstrated only for some of the species *R. gallica, R. pendulina* and *R. zalana*. However for *R. rubiginosa*, which has the highest viability of pollen within section *Caninae* (TTC test: 54.56%), the germination rate no higher 21%. In most of the examined species the apparent viability of pollen grains was higher than their capability of germination. The lower ability of germination could have been related to with insufficient *in vitro* conditions (Stanley and Linskens 1974). The results of the studies on germination and viability of pollen grains in some of the species of section *Caninae* by other authors are not explicit. Some of them report about their weak viability, which according to Werlemark (2000) is 22.4% (*R. dumalis*) and 26.4% (*R. rubiginosa*). Ueda and Akimoto (2001) estimated in them in the range of 23–45%. Such similar results of TTC test (31–47.24%) for *R. dumalis* and *R. villosa* genotype have obtained Ercisili (2007). In his researches, two *R. dumalis* genotypes had higher pollen viability (47.24; 43.15%), than *R. villosa* genotypes (33.9; 31.8%). While Günes et al. (2005), using the same TTC test for *R. canina, R. dumalis, R. inodora, R. rubiginosa* and *R. villosa* genotypes, obtained much higher results of pollen viability (66.2–87.9%). On the other hand Ji inska et al. (1976), who examined pollen viability of eight *Rosa* species from three sections (*R. arvensis*, *R. canina, R. gallica, R. jundzillii, R. majalis, R. pendulina, R. pimpinellifolia* and *R. ×reversa*) using also TTC test obtained more diverse values (from 14.8% in *R. canina* to 97.3% in *R. majalis*).

The results of our studies confirm the conclusions of Ji inska et al. (1976), and indicate that the pollen viability of examined species of section *Caninae* varies. In our examinations, pollen viability using TTC test indicated values from 29.43% in *R. agrestis* to 54.56% in *R. rubiginosa*. For *R. canina* pollen viability had a much higher value (38.34%) compared with Ji inska et al. (1976). The most viable pollen seemed to have *R. rubiginosa* and *R. dumalis*. The first one has values of 46.06% (Müntzing test), 54.56% (TTC), 87.58% (FCR), the former presents as following: 48.26% (Müntzing test), 32.56% (TTC) and 62.24% (FCR). While *R. villosa, R. jundzillii* and *R. inodora*, belong to the species of which have the weakest pollen viability: *R. villosa* – 33.9% (TTC) and 46.81% (FCR); *R. jundzillii* – 15.51% (Müntzing test), 50.52% (TTC) and 49.05% (FCR); *R. inodora* – 25.35% (Müntzing test) and 37.1% (FCR). Ji inska et al. (1976) have reported the lower values of viability in TTC test for *R. pendulina* and *R. gallica*; for *R. pendulina* from 77.9 to

79.8%, whereas in our examination the mean values were 90.05% and for *R. gallica* from 57.8 to 79.1% and our results were mean value of 92.67%.

In our studies we use the FCR test for the first time to examine pollen viability of *Rosa*. We obtained the highest mean values of pollen viability of all examined species (59.82%), about 10% higher than the other tests. Comparing the TTC test for particular species with FCR, we usually obtained higher values of viability in 9 of 14 species in favour of FCR. In *R. dumalis, R. rubiginosa* or *R. agrestis* the FCR test values were about 30–36% higher than for TTC. In species such as *R. mollis, R. jundzilli, R. sherardii* and also *R. pendulina* and *R. gallica*, the TTC test values were higher than FCR, in the case of *R. mollis* about 15% more. It is difficult to clearly interpret this result because the FCR test was used for the first time, so there is no comparative data. In our study included only a properly constructed pollen, although some of them were less saturated with the FDA and, therefore, less shone, however, it did not pose a problem in the calculation of the percentage of viable pollen grains. In further studies using the FCR test to evaluate the viability of pollen, it would be to focus on the problem how many deformed pollen, which are very poorly saturated with FDA and light very weakly or not at all, can be ignored in the assessment of viability.

The pollen viability tested after 6 month storage in deep freeze (–25°C) using Müntzing procedure for part of species (*R. micrantha, R. mollis, R. villosa*) was 10–13% higher than before storage, for *R. zalana* even in 26% higher than tested soon after dusting ripe pollen (Table 1). For *R. gallica* and *R. jundzillii,* viability is very similar; only for *R. rubiginosa,* pollen viability turned out to be significantly lower (at about 30%) than before storage. It can be concluded, that low temperature has good influence on viability of pollen in most of the examined species. This phenomenon has been confirmed by the studies of the other authors. Rajasekharan and Ganeshan (1994) report that after one year cryopreserved pollen (liquid nitrogen, –196°C) was comparable to fresh pollen for both pollen germination using an *in vitro* assay. When rose pollen was stored at warmer temperatures (–24 to 25°C); however, pollen viability declined within a year (Khosh-khui et al. 1976; Visser et al. 1977). Wang et al. (2009) examined the effect of pollen storage in temperature of –4 and –6° C for the viability of some cultivars of Pingyin rose. In their opinion the viability of pollen in natural conditions decreased very rapidly. Thanks to freezing, the pollen viability decreased slowly and maintain on relatively high level even after 60 days after storage. Numerous pollen grains of the of Pingyin rose cultivars have kept viability even one year after frozen storage.

Ji inska et al. (1976) reported that pollen grains germinability remained in good correlation with the TTC test. In this study, this relationship was corroborated only in a small number of species (e.g. in *R. gallica, R. pendulina* and *R. zalana*).

According to Stanley and Linskens (1985) and Westwood (1978) the most important substance is sucrose, which has a two-fold effect; it regulates the osmotic pressure of the pollen grain and it is used as a nutrient for the pollen tube growth. For *in vitro* experiments, addition of external minerals and growth regulators such as boric acid, calcium nitrate, potassium nitrate, magnesium sulphate and gibberellic acid can stimulate pollen germination and pollen tube growth. The results obtained by Ji inska et al. (1976) showed that the optimal concentration of sucrose in agar was rather high in all cases, about 20% for *R. canina, R. jundzillii, R. pendulina, R. pimpinellifolia* and *R. ×reversa* and about 30% for *R. majalis*. Pollen germinability of *R. arvensis* and *R. gallica* was similar, in the range of sucrose concentrations in agar (10–30%) with optimal concentration of 20–25%. The research results of many scientists confirm that sucrose concentrations of 15 and 20% are reported as the best media for pollen germination not only in roses, but also in many different species or cultivars (Täckholm 1922; Mameli Calvino 1951; Visser et al. 1977; Choudhary 1990; Eti 1990, 1991; Ak et al. 1993; Eti et al. 1994). Koncalova (1975) reported higher pollen germination percentage of *R. hugonsis* at 30 and 35% sucrose concentrations. Identical results were obtained by Ercisli (2007) for *R. dumalis* genotypes (35% sucrose) and *R. villosa* genotypes (30% sucrose). Voyiatzi (1995) claimed that pollen grains of rose cultivars germinated poorly in a medium containing sucrose only. Addition of boric acid in the medium improved germination percentages. This is corroborated by the results of investigations performed by Mameli Calvino (1951), Visser et al. (1977) or Ueda and Hirata (1989). The latter researchers examined several dozen *Rosa* taxa from the angle of the dependence of pollen germination on 1% agar medium with 10% sucrose on boric acid concentrations (0, 50 or 100 ppm) and on temperature (20, 25 and 30°C). They stated that temperature did not influence on pollen germination significantly, but the concentration of boric acid. Pollen of wild roses species at the temperature of 30°C on agar media without the addition of boric acid germinated more poorly (e.g. 15.3%) in comparison with the media with 50 or 100 ppm boric acid (43.5; 44.5%, respectively). According to Voyiatzi (1995), the highest germinability was observed on 15% sucrose+50 ppm boric acid and 20% sucrose+100 ppm boric acid, respectively. Observations of the above researcher were corroborated by Günes et al. (2005) according to whom, the optimal medium for *R. dumalis, R. inodora, R. canina, R. villosa* genotypes is agar 1%+15% sucrose+50 ppm boric acid as it was then that germination from 54% (in ge-

notype 2 – *R. inodora*) to 76.6% (genotype 3 – *R. canina*) was obtained. Also agar 1%+15% sucrose and agar 1%+15% sucrose+100 ppm boric acid turned out to be good media.

Also in our studies, the highest germinability was observed on 15% sucrose+50 ppm boric acid (up to 39.93% in *R. pendulina*) and agar 1.5%+15% sucrose+50 ppm boric acid (up to 42.56% in *R. zalana*). However, according to our findings, the impact of the addition to the media of 50 ppm boric acid on pollen germination was not unambiguous. In the case of some media, it improved germination but in the case of others, it failed to exert any impact or even decreased it (see: Table 2).

Voyiatzi (1995) reported that an addition of calcium nitrate to the medium reduced germination percentages in all cultivars. This result was corroborated partially by Sýkorová et al. (1976) who maintain that following the addition of calcium in the germination media, the rose pollen germinated in lower sucrose concentrations, but the optimum ranges of sucrose concentration were in both cases very similar with regard to the percentage of germination. The above-quoted researchers also analysed the effect of the medium pH on pollen grain germinability depending on the cultivar and the composition of the medium. On a medium free of boric acid, the pH values between 5.5 and 7.0 caused a significant increase in the germination percentages of pollen grains of two cultivars. The addition of boric acid decreased the responsiveness of the pollen of all cultivars to pH changes of the medium.

Günes et al. (2005) were the only ones who investigated germination of pollen grains of the studied roses after the addition to the agar medium (1% agar+15% sucrose), 100, 200 and 400 ppm of gibberellic acid. It turned out that at 100 ppm gibberellic acid, all genotypes germinated – albeit at a low level – at 200 ppm, only genotype 3 (*R. canina*) germinated and at 400 ppm, there was no germination in all genotypes.

In this study, also for the first time, pollen germination levels of three agar media with 12% maltose and 12% lactose with or without 50 ppm boric acid were tested but they failed to turn out to be optimal for any of the examined species. The majority of them did not germinate despite the fact that their pollen was alive or germinated on a low or very low level as in the case of *R. zalana, R. canina* and *R. villosa* (respectively: 15.95; 13.82; 3.33%). The only exception was *R. gallica* which germinated on the last two media at a fairly high level (35.92 and 43.08%, respectively).

Conclusions

– The linear correlation between pollen viability and germination suggested by some researchers was corroborated only in some cases (e.g. in *R. gallica, R. pendulina* and *R. zalana*) but viability of pollen grains in the majority of the examined species was higher than their germination rate.
– Species from sections *Gallicanae* (*R. gallica*) and *Rosa* (*R. pendulina*) revealed a very high and similar level of viability (mean: 90.69% and 86.85%, respectively), and medium level of germination capacity (mean: 31.11% and 35.85%, respectively). They confirm the same, their taxonomical position of "good species". The mentioned species are distinctly different from the majority of studied species from section *Caninae*, in which we found a lower and more varied level of viability, what in turn confirms the hybrid nature of the representatives of this section (Tables 1 and 2).
– The examined pollen grains germinated most numerously on agar medium with 1.5% agar+15% sucrose+50 ppm boric acid (11 out of 13 examined species) and liquid medium with 15% sucrose+50 ppm boric acid (9 out of 13 examined species). The optimal liquid and agar media were those in which sucrose concentration was 15% and which were supplemented with 50 ppm boric acid. Liquid media with 10 and 20% sucrose and agar media with 10% sucrose+50 ppm boric acid also turned out to be quite good.
– *R. gallica* turned out to be a "universal species" whose pollen grains viability and germination on all test's and media are usually on a very high level (up to 92.67% and up to 63.4%, respectively; Table 1, 2).
– Following deep-freeze storage (–25°C) for the period of 6 months, the pollen grains of the selected 9 species (with the exception of *R. rubiginosa*) showed viability higher or similar to that before storage.

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