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ROMANIAN KIWIFRUIT BREEDING PROGRAM - PRELIMINARY STUDY OF FIFTEEN MALE HYBRIDS FOR SELECTION AS POLLINATORS

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Abstract
Kiwi is a new fruit that can be grown in Romania. A common Italian-Romanian kiwifruit breeding program was initiated in 1993. For pollinator (male) kiwi plants, breeding programs involve the selection of elite plants with long flowering period and high pollen germination rate. The aim of this study was to evaluate the pollen grains from fifteen Romanian kiwifruit hybrids for identifying the most suitable pollinators for kiwi female selections released from our breeding program. The fresh pollen grains were collected during pollination period (2018-2020), in Petri dishes, maintained at room temperature for 12 hours, and then placed in 15% sucrose solution for germination. At different time interval: 4, 8, 12 and respectively, 24 hours, several measurements were done. Four genotypes – R0P3, R0P6, R2P8 and R3P9, which recorded over 90% germinability after 24 hours, have been selected for further field tests, including artificial pollination and compatibility tests with the kiwi female selections.

Keywords: Actinidia deliciosa, Actinidia chinensis, pollen germination, pollen tube growth, sucrose solution

1. INTRODUCTION

The genus *Actinidia* is native to the humid highlands of south-western China and has spread out to several regions of the world (Ferguson, 2007) regarding his rich nutritional composition of the fruits (Huang et al., 1997). The biochemical properties of the kiwifruit and it's high vitamin C content made it popular among consumers as a healthy fruit (Li et al., 2007). The genus contains more than 50 species and most of them are functionally dioecious, with pistillate and staminate flowers occurring on the separate plants (Ferguson, 1990; Borghezan et al., 2011). Therefore, the fruit set is mostly affected by its own flower biology (Costa, 1993; Snowball, 1996).

Both male and female flowers have both sexes, but staminate vines produce male flowers that contains viable pollen, without functional ovaries and pistillate vines produce female flowers with a functional ovary and non-viable pollen (Schmid, 1978; Howpage et al., 1998; Delaplane and Mayer, 2000). Pistillate flowers are typically larger than staminate flowers, but male vines usually carry more flowers according to Devi et al. (2015).

Pollen grains are small (Goodwin, 1987) and are shed abundantly in clumps from both pistillate and staminate flowers (Schmid, 1978). According to literature, male flowers produce larger quantities of

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pollen grains that insects carry to the stigmas of female flowers (Schmid, 1978; Howpage et al., 1998; Delaplane and Mayer, 2000).

Although flowers are scented, particularly staminate flowers, they are not capable to produce nectar (Schmid, 1978). So, besides insects (especially bees), the wind also contributes to pollination.

Results confirmed that the yield and postharvest quality of fruits are strongly affected by an effective pollination (Stănică and Hoza, 1992; Goodwin et al., 2013). Fertilization of a large proportion of ovules results in bigger kiwifruit (Hopping, 1976). Therefore, fruit size and seed numbers are positively correlated (Pyke and Alspach, 1986; Testolin et al., 1991).

Insufficient pollination leads to unsatisfactory fruit size, shape and uniformity, which, in turn, reduces the market value of the production (Goodwin, 2000). Fruit size is the main factor that determines quality, and together with a proper orchard management, optimal production can only be achieved if conditions for pollination and fruit set are the best (Tromp et al., 2005).

For all these reasons, pollination is a very important and inseparable component in respect of regular and consistent production in a number of fruit crops (Hopping et al., 1982; Costa et al., 1993; Underwood, 2001; Petrisor et al., 2012). The viability, tube growth and morphological homogeneity related to pollen quality are the most important properties in fruit plants (Petrisor et al., 2012). These properties are useful for plant breeders, geneticists, and growers (Bolat and Pirlak, 1999). Relationships between viability and pollen germination have been studied and positive correlation between them was reported by Werner and Chang (1981), Pearson and Harney (1984).

Starting with 1970, according to Davison (1974); Palmer and Clinch (1974), studies of floral biology and pollination of kiwifruit began appearing. Numerous studies on pollen grains have continued and addressed reproductive physiology (Falasca et al., 2010), conservation of germplasm (González-Benito et al., 2004), breeding (Cruz et al., 2008; Chagas et al., 2010; Novo et al., 2010), pollination and fruiting (Nunes et al., 2001; Bettiol Neto et al., 2009). For male kiwi plants in breeding programs, it is important to have ability to produce viable pollen and the flowering periods it is necessary to coincide with the tested fruit producing varieties (Novo et al., 2010).

Several methods can be mentioned for the evaluation of pollen viability: using artificial sucrose media - "Sitting Drop" culture method (Shivanna and Rangaswamy, 1992); evaluation by acetic carmine staining (Domingues et al., 1999); incubation in Baker solution (Oliveira et al., 2001) or the germination test in culture medium (Abreu and Oliveira, 2004; Einhardt et al., 2006; Franzon and Raseira, 2006; Pio et al., 2007).

In Romania, kiwifruit was introduced in 1993, in the South East area through an Italian-Romanian kiwifruit breeding program (Stănică and Zuccherelli, 2007; Stănică, 2009; Stănică and Zuccherelli, 2009). After more than two decades of research, several hybrid genotypes were obtained and introduced to be tested. For pollinator (male) kiwi plants, breeding programs involve the selection of elites with high pollen germination and long flowering period. In previous research (Cotruț et al., 2014) germination rate of few Romanian kiwi genotypes (*Actinidia spp.*) was evaluated after 3, 6 and 9 hours in a culture medium containing 20% sucrose, 5 ppm boric acid (H3BO3) and 1% agar. The results showed that in all kiwi genotypes the germination rate and pollen tube growth varied according to the incubation period and most of the studied genotypes appear to be suitable pollinators.

The aim of this study was to evaluate the pollen grains quality of fifteen kiwifruit hybrids express by: shape index of viable and dead pollen grains, viability percentage (%), germination rate (%) and pollen tubes length (μm) after 4, 8, 12 and 24 hours. The results were used to identify the most suitable pollinators for kiwi female selections released from our breeding program.

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2. MATERIALS AND METHODS

During 2018-2020 pollination period (early May to beginning of June), flowers from fifteen kiwifruit hybrids (*Actinidia spp.*) were collected. The biological material studied was represented by 12 male plants and 3 hermaphrodite ones, some of them are presented in Figure 1. The plants were organically grown in Southern Romanian climate, in the Experimental Field of the Faculty of Horticulture, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, and were trained on a T-bar trellis system.

Figure 1. Male flowers from kiwifruit hybrids (Actinidia spp.)

Anthers from picked flowers were kept in Petri dishes, in the laboratory at room temperature, for 12 hours (Cociu and Oprea, 1989). After the anthers dried and dehisced, the obtained pollen grains were placed in a 15% sucrose solution for germination (Mangalore et al., 2017). The solution was prepared by dissolving 15 grams of sucrose in 100 ml of distilled water (Figure 2).

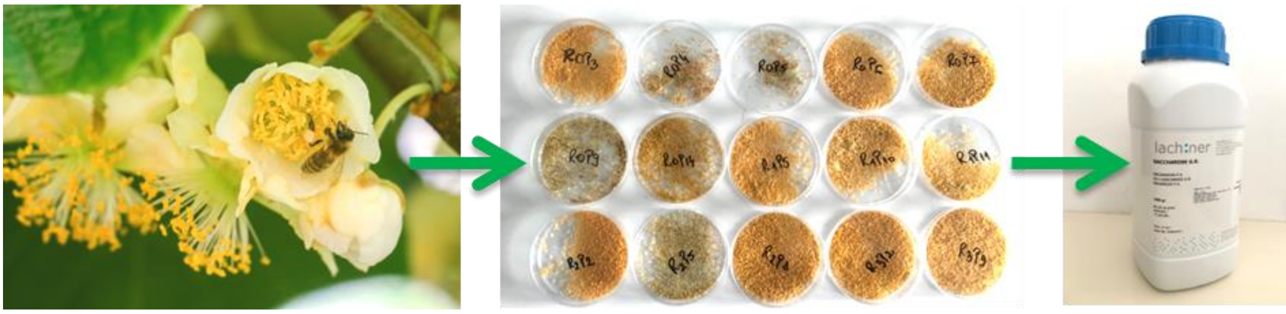

Figure 2. Anthers from picked flowers kept in Petri dishes and samples preparation steps

After 4, 8,12 and respectively 24 germination hours, several measurements (pollen grains length and width; pollen tube length) and observations (pollen viability; germination rate) were made with Leica DM 1000 LED Microscope equipped with Leica DFC 295 Camera and LAS Core software (Figure 3). Length and width were measured for both viable and dead pollen grains, and expressed in µm. Also pollen tube length was expressed in µm. Germination rate was expressed as a percentage and was calculated by dividing the number of germinated pollen grains per view field by the total number of pollens per view field (Mangalore et al., 2017).
The pollen viability was expressed as a percentage and was determined as the sum of all viable pollen grains per view field divided by the sum of all pollen grains per view field (Mangalore et al.,

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2017). Dead pollen grains were recognized because of the darker colour and smaller dimension than viable pollen grains (Stănică and Hoza, 1992; Mangalore et al., 2017).

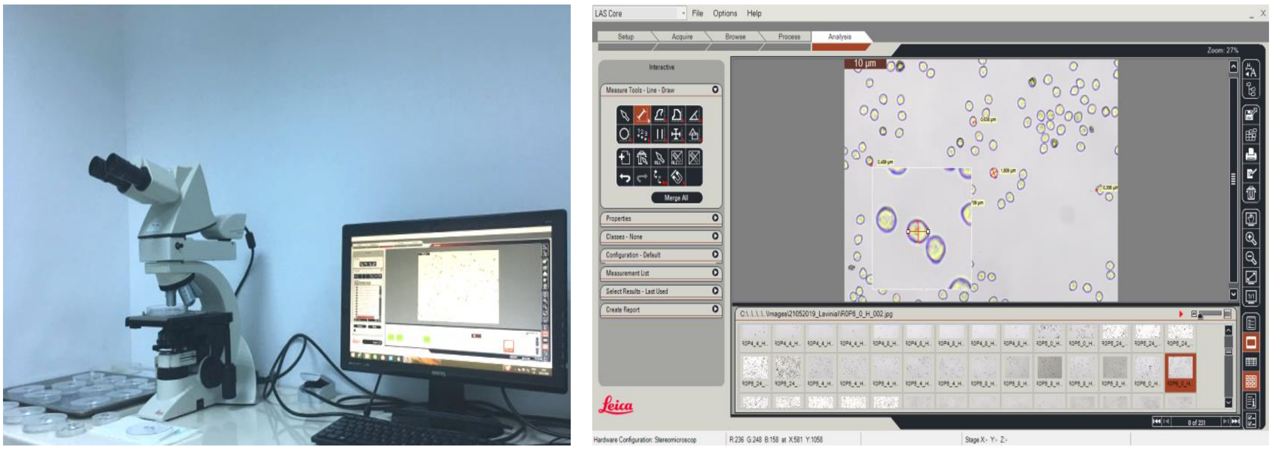

Figure 3. Leica DM 1000 LED Microscope equipped with Leica DFC 295 Camera and LAS Core software

The average germination percentage and viability percentage for each kiwifruit hybrid was calculated from five replications (five different overviews from the microscope slide). At least thirty pollen grains were chosen randomly to determine the mean tube length, the mean length and width of viable and dead pollen.

3. RESULTS AND DISCUSSIONS

The plants evolution growth stages developed differently depending on the temperatures and climatic conditions recorded in every year. So, the BBCH 60-69 stages, were registered with a delay of about 1-2 weeks, in 2019 compared to 2018 and 2020. The flowering period ensued between the first decade of May and the first decade of June, for most hybrids (Figure 4). The genotype with the earliest flowering was R0P7, and the longest flowering period was recorded for the hybrid R2P8.
The flowering period of the kiwi plants is quite long, 3-5 weeks, because not all flowers bloom at the same time, especially for varieties with several flowers in inflorescence. It is known that the stigma of female flowers is viable for 7-9 days (Sale, 1981; Goodwin, 2000) from the beginning of flower opening, so a prolonged flowering of male varieties helps to better fertilize the ovaries.
The kiwi fruits size and weight at harvest depend especially on the number of seeds fertilized during pollination period (Stevens and Forsyth, 1982; Anon, 1983; Woolley et al., 1988). Also, the fruits size and weight are affected by management factors such as irrigation, fertilization, the ratio between vegetative growth and the number of fruits, the plantation microclimate etc. (Clinch, 1984; Lees, 1986; Sale, 1986; Lawes et al., 1990; Patterson et al., 1999; Goodwin, 2000; McPherson et al., 2001). The number of seeds is in turn closely related to the number of viable pollen grains deposited on the stigma, so the selection of male plants with a long flowering period and a high quality of germination of pollen grains is a priority among breeding programs (Hopping, 1981; Hopping, 1985; Gonzalez et al., 1994).
Studying the pollen grains shape, in Table 1 can be observed that most of them are ellipsoidal, as Schmid (1978), Dickison et al. (1982), Ferguson and Pusch (1991) mentioned in their research.

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According to previous studies, dead pollen grains are smaller that viable ones (Korkutal et al. 2004; Devi et al., 2015) and our results confirmed so (Table 1). In Figure 5 can be observed that dead pollen grains have a darker color compared to viable ones.

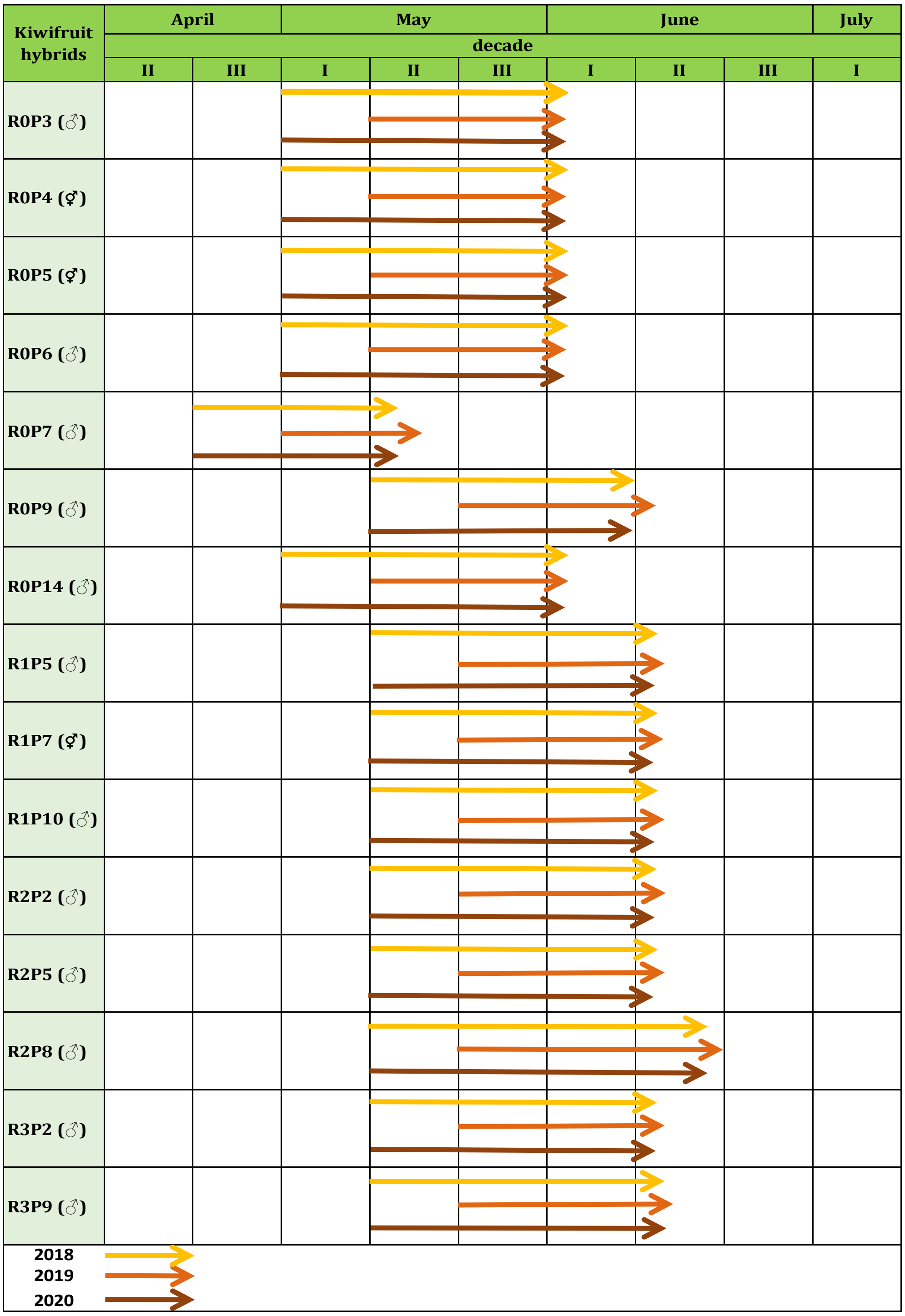

Figure 4. The flowering period of the studied hybrids

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Table 1. Kiwifruit pollen grains measurements (µm)

Kiwifruit hybrids	**Viable pollen grains**		**Dead pollen grains**	
	length (µm)	**width (µm)**	**length (µm)**	**width (µm)**
R0P3 (♂)	1.864 ± 0.252	1.573 ± 0.211	1.596 ± 0.112	0.920 ± 0.081
R0P4 (⚥)	2.021 ± 0.296	1.758 ± 0.296	1.927 ± 0.227	1.103 ± 0.094
R0P5 (⚥)	2.172 ± 0.293	1.841 ± 0.327	1.941 ± 0.317	1.339 ± 0.305
R0P6 (♂)	1.849 ± 0.242	1.740 ± 0.153	1.742 ± 0.286	1.474 ± 0.417
R0P7 (♂)	1.969 ± 0.147	1.722 ± 0.143	1.704 ± 0.376	1.212 ± 0.042
R0P9 (♂)	1.741 ± 0.122	1.154 ± 0.259	1.659 ± 0.272	0.930 ± 0.037
R0P14 (♂)	1.957 ± 0.134	1.356 ± 0.298	1.569 ±0.186	1.084 ±0.124
R1P5 (♂)	2.017 ± 0.227	1.623 ± 0.173	1.011 ± 0.124	0.780 ± 0.100
R1P10 (♂)	2.109 ± 0.124	1.756 ±0.056	1.525 ± 0.347	0.912 ± 0.047
R2P2 (♂)	1.884 ± 0.163	1.508 ± 0.149	1.474 ± 0.232	1.048 ± 0.098
R2P8 (♂)	1.970 ± 0.171	1.751 ± 0.163	1.893 ± 0.137	1.376 ± 0.122
R3P2 (♂)	1.874 ± 0.116	1.633 ± 0.157	1.815 ±0.149	1.240 ± 0.028
R3P9 (♂)	1.774 ± 0.169	1.639 ± 0.219	1.674 ± 0.197	1.294 ± 0.120

Note: Mean values ± Standard Deviation

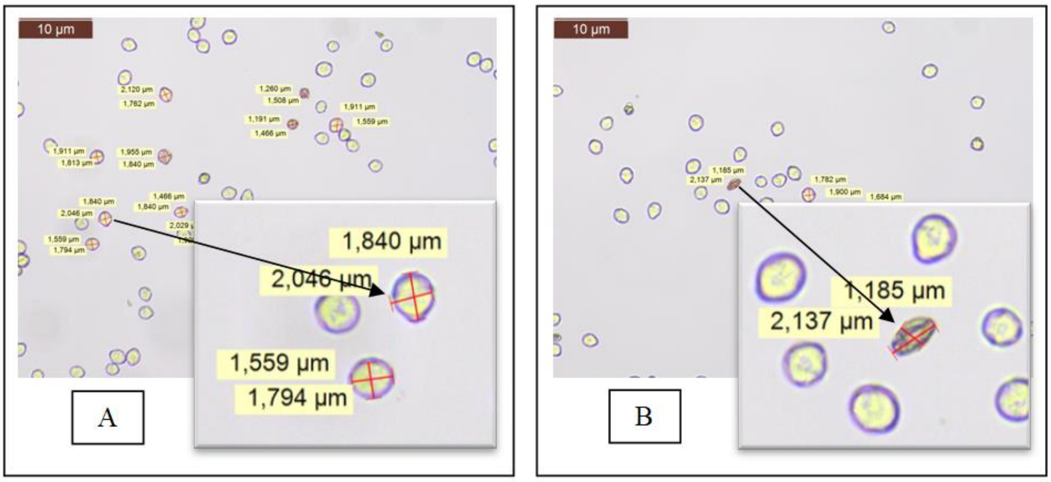

Figure 5. Viable (A) and dead (B) pollen grains of R0P7 male kiwifruit hybrid, after immerging in a 15% sucrose solution

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In Figure 6, can be observed that all studied hybrids recorded over 90% pollen viability, except the hermaphrodite ones R0P4 and R0P5, which recorded 72.48 % and respectively 65.34 %, in 2019, and 64.49 % and respectively 57.47 %, in 2020.

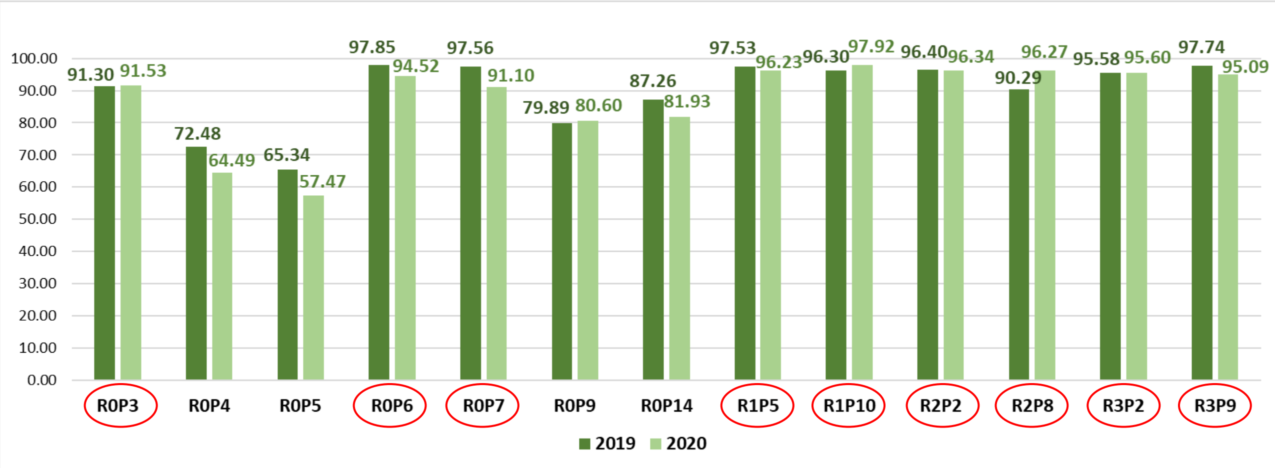

Figure 6. Kiwifruit hybrids pollen viability rate, 2019-2020

According previous studies (Werner and Chang, 1981; Pearson and Harney, 1984), a positive correlation between pollen viability and pollen germination can be observed. For all studied hybrids, pollen germination rates were gradually increasing after 4, 8, 12 and 24 hours. The highest values of the germinability rate, after 24 hours, were recorded at hybrids R2P2 – 90,27%, R0P3 – 90.79 %, R0P6 – 91.17 %, R1P5 – 91,22%, R1P10 – 91,96%, R3P9 – 93.23 % and R2P8 – 93.35 % (Figure 7).

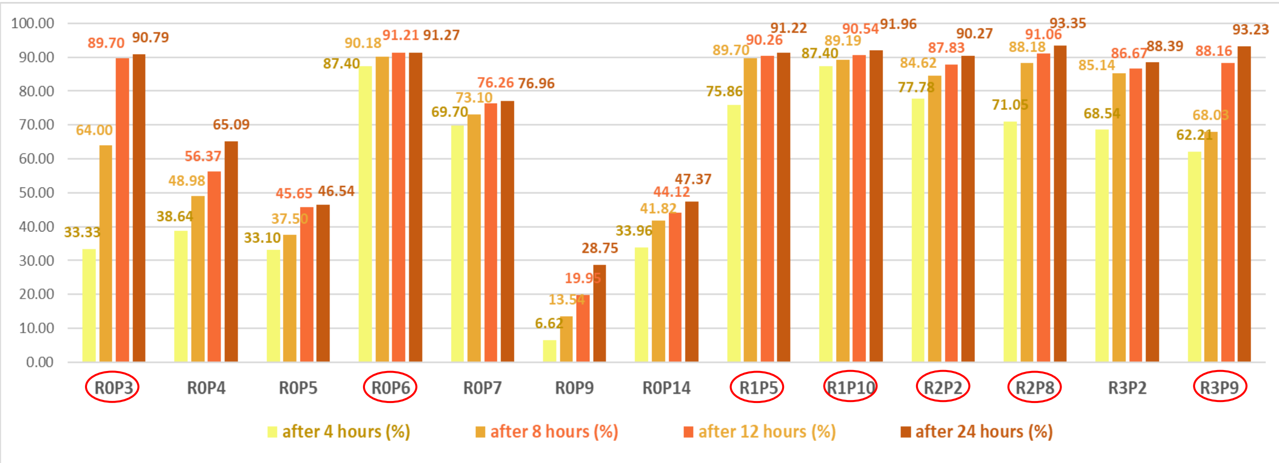

Figure 7. Kiwifruit hybrids pollen germination rate at different time intervals

The results showed that pollen germination rate and pollen tube length were strongly influenced by the genotype and varied also according to the incubation period. Pollen tube lengths for each hybrid increased in time, after 4, 8, 12 and respectively 24 hours (Table 2). After 24 hours of germination, the highest values of pollen tube length were recorded at R3P9 (♂) – 5.440 µm, flowed by R2P8

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(♂) – 4.805 µm, while the lowest value was found at R0P4 (⚥) – 1.460 µm. The standard deviation showed significant differences between pollen tube lengths in the same view field for all genotypes (Figure 7).

Table 2. Kiwifruit hybrid pollen tube length (mean ± SD in µm) during 24 hours

Kiwifruit hybrids	Pollen tube length after			
	4 hours (µm)	8 hours (µm)	12 hours (µm)	24 hours (µm)
R0P3 (♂)	1.713 ± 0.952	2.327 ± 0.455	2.489 ± 0.617	4.206 ± 0.978
R0P4 (⚥)	0.834 ± 0.396	1.197 ± 0.410	1.364 ± 0.440	1.460 ± 0.876
R0P5 (⚥)	1.227 ± 0.776	1.384 ± 0.545	1.455 ± 0.470	2.977 ± 2.056
R0P6 (♂)	0.745 ± 0.328	1.250 ± 0.538	1.397 ± 0.563	1.754 ± 1.623
R0P7 (♂)	0.814 ± 0.246	0.818 ± 0.427	1.351 ± 0.346	1.659 ± 0.736
R0P9 (♂)	0.265 ± 0.002	0.903 ± 0.614	1.121 ± 0.233	1.626 ± 0.137
R0P14 (♂)	0.627 ± 0.316	0.781 ± 0.531	1.411 ± 0.008	1.681 ± 0.085
R1P5 (♂)	1.395 ± 0.593	1.808 ± 0.604	1.928 ± 0.823	2.398 ± 0.889
R1P10 (♂)	1.514 ± 0.093	1.526 ± 0.266	1.656 ± 0.079	1.781 ± 0.479
R2P2 (♂)	1.388 ± 0.532	1.879 ± 0.886	2.682 ± 0.954	3.396 ± 0.960
R2P8 (♂)	1.291 ± 0.441	1.388 ± 0.542	2.320 ± 0.701	4.805 ± 0.834
R3P2 (♂)	0.977 ± 0.540	1.681 ± 0.764	2.343 ± 0.870	4.018 ± 0.794
R3P9 (♂)	1.593 ± 0.605	1.627 ± 0.690	1.908 ± 0.579	5.440 ± 0.816

Note: Mean values ± Standard Deviation

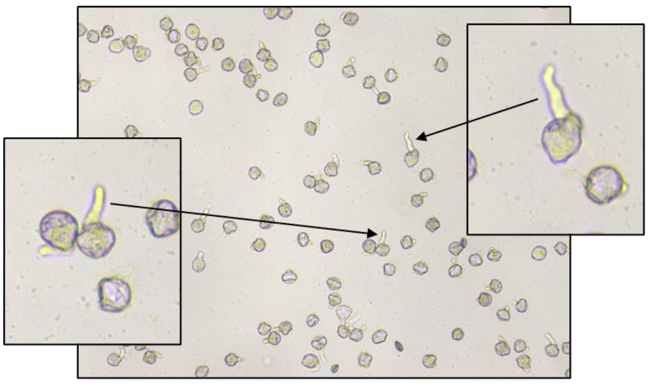

Figure 7. Pollen tube length of R2P8 kiwifruit male hybrid after 8 hours of germination

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In the present study we found out that the kiwifruit pollen registered the highest germination rate after 24 hours, using 15% sucrose solution (according also with the previous studies – Mangalore et al., 2017).

4. CONCLUSIONS

Regarding the study of male plants flowering period, it can be mentioned that most kiwi hybrids bloomed between the first decade of May and the first decade of June. The R0P7 hybrid ensued the BBCH 60 stage in the last decade of April, with the earliest development and the longest flowering period was recorded for the R2P8 hybrid.

Results showed that in all kiwi genotypes the germination rate and pollen tube growth varied according to the incubation period. The highest percentage of germination (93%) was recorded after 24 hours of incubation for R2P8 and R3P9. Because the evaluation of pollen germination rate is an essential criterion for kiwi pollinator's characterization, from the eight male hybrids, four genotypes – R0P3, R0P6, R2P8 and R3P9 (which recorded over 90% germinability rate after 24 hours), have been selected for further field tests. Besides artificial pollination and field compatibility tests with the female kiwifruit plants, other ploidy studies have to be done. Most of our kiwifruit hybrid genotypes have been obtained from interspecific crosses (*A. deliciosa* x *A. chinensis*) and probably have different ploidy laves. For a good pollination rate, male and female plants need to have the same ploidy levels. In order to match the right level of ploidy for male-female couples, further studies using the flow cytometry have to be completed.

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