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Study on Pollen Viability of *Rubus* Spp. at Cibodas Botanic Gardens

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Abstract. *Rubus* spp. known as raspberries is a belonging genus of the Rosaceae. *Rubus* have a wide range of medicinal and nutritional property, but it is rarely cultivated for commercial need in Indonesia. It is due to a lack of information about Indonesian *Rubus*. The information about flowering and pollen viability will contribute to its cultivation efforts and increase the success rate of hybridization. The aim of this study was to determine the pollen viability of *Rubus* spp. collection of Cibodas Botanic Garden. Ten species of *Rubus* spp., which are 8 species of wild type and 2 species commercials, were tested for pollen viability. The pollen viability test was observed by the in vitro germination and staining method. The result showed that the viability of pollen grains ranged from 59.68 % to 98.12%. *Rubus linneatus* showed the fastest and highest pollen germination in 1-hour germinated. On the other hand, the slowest of pollen germination was Blackberry, with 1.7 % after 1 hour germinated. There was a positive correlation among germination, pollen viability, and pollen tube lengh.

INTRODUCTION

Rubus (*Rubus* Spp.) is a group of fruit plants belongs to the Rosaceae family, and it has a high diversity of species with a fairly wide distribution area. *Rubus* can be found in almost all continents, both in the highlands and lowlands except Antarctica [1]. Kalkman [2] reported that *Rubus* was found at an altitude of 1000 - 3000 m asl between the sub-mountain zone with the sub-alpine zone, but several species also found to grow below 1000 m asl and above 3000 m asl. Moreover, 25 species of *Rubus* is widely distributed in the highlands, lowlands, and mountain forests of Indonesian islands [3,4,2,5].

Rubus has a high ecological and economic potential, and it is one of the pioneers of succession in the forest. On the other hand, *Rubus* can be used as fruit crops, ornamental plants, and medicinal plants [6]. Commercial Rubus is widely found in several countries, including America, Australia, Germany, such as raspberries (*Rubus idaeus*), blackberries, and dwarf raspberries (*Rubus pubescens*). However, in Indonesia, information about the cultivation and utilization of Indonesian *Rubus* is still limited, thus provides a great opportunity for domestication and commercialization as fruit crops by increasing the quality of plants through plant breeding. Futhermore, knowledge

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of flowering and pollen viability will contribute to the cultivation efforts and increasing the success rate of hybridization [7].

Cibodas Botanic Gardens (CBG), as an ex-situ conservation institution, has *Rubus* as garden collections. They namely *Rubus acuminatissimus, Rubus alpestris, Rubus alceifolius, Rubus chrysophyllus, Rubus ellipticus, Rubus elongatus, Rubus fraxinifolius, Rubus lineatus, Rubus moluccanus, Rubus rosifolius, Rubus sumatranus, Rubus pyrifoliu*s, and *Rubus* sp. Besides the 13 species of *Rubus,* the CBG also collects commercial cultivar such as raspberry and blackberry. Even though several research activities on Indonesian *Rubus* such as flowering and fertilization [8], breeding [9,10], propagation, and cultivation [10,11,12,13] have been carried out, however, information about pollen viability of R*ubus*, especially for Indonesian still need to be studied. The pollen viability is known affected by the reproduction and breeding program of *Rubus*. Therefore, the aim of this study was to investigate reproductive biology and pollen viability of *Rubus* collection of CBG, which is implicating on breeding systems and its life cycles.

MATERIALS AND METHODS

The experiment was carried out at Cibodas Botanic Gardens, West Java, from February – July 2019. *Rubus* collections of CBG, which are tested for pollen viability, are consist of ten species; eight species of wild type, namely *R. chrysophyllus*, *R. ellipticus*, *R. fraxinifolius*, *R. rosifolius*, *R. linneatus*. *R. pyrifolius*, *R. moluccanus, R. alcefolius*, and two commercial species Raspberry and Blackberry.

The pollen grain was grown in Brewbaker & Kwack (BK) media (10% sucrose + 100 ppm H_3BO_4, 300 ppm $Ca(NO_3)_2.4H_2O$, 200 ppm $MgSO_4.7H_2O$, 100 ppm KNO_3 in 1000 ml distilled water) [14]. The staining method using 1% I_2KI solution was used to observe pollen viability. The germination and pollen viability were observed in four different times (1, 3, 6, and 24 hours). The pollen germination, viability, pollen area, and pollen tube length were the four parameters recorded using a microscope at 100x magnification equipped with a micrometer on Optilab software. Pollen germination was calculated by dividing the number of germinated pollen grains with a total number of pollen grains observed. Pollen viability was calculated by dividing the number of stained pollen grains by a total number of pollen grains observed. Viable pollen is marked with a darker pollen color, while pollen that is not viable (sterile) looks transparent [15]. The studies were done in the randomized design with three replications for each species and interval time. The Spearman rank correlation was used to see the correlation between pollen germination, pollen viability and tube length.

RESULTS AND DISCUSSION

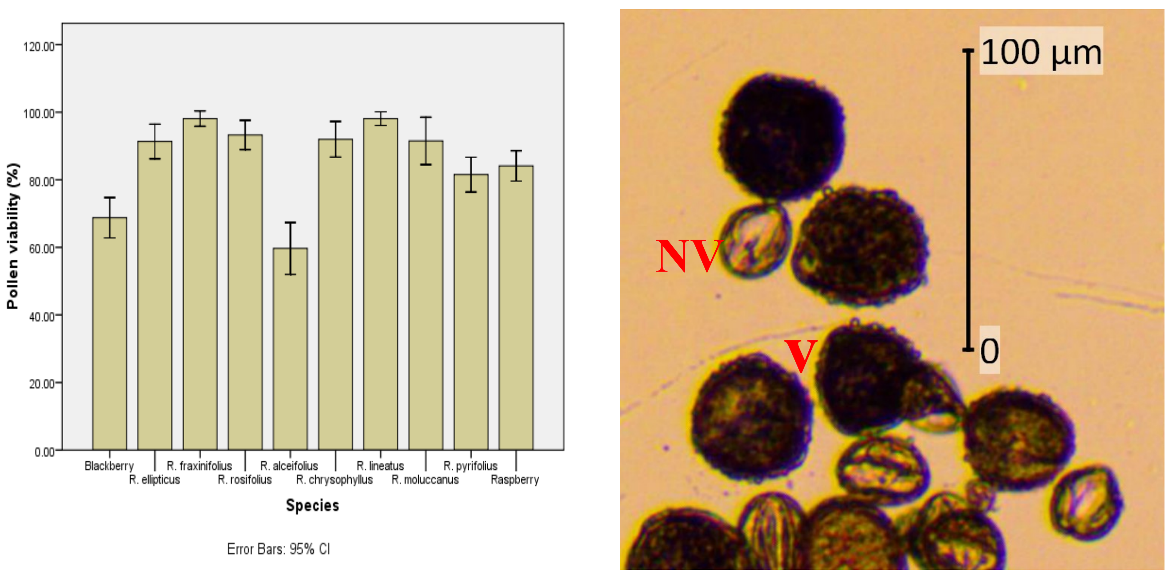
Pollen viability

The viability of pollen grains was estimated by the staining method using 1% I_2KI solution. The results (Table 1 & Figure 1.a) indicated that *Rubus* has a varying level of pollen viability, ranged from 59,68 % to 98.11 %. Various levels of pollen viability percentage in this experiment indicate that there are genotype differences among the *Rubus* species. This finding similar to that reported by Nybom, who noted genotype differences in blackberry species [16], and in *Prunus avium* [17]. The staining method is a pollen viability test, which is based on the assumption of nutrient content contained in pollen. If the nutrient were sufficient, it would be considered viable [18].

The pollen grains that intensely stained when observed under the microscopic field were counted as viable, whereas poorly stained were considered as non-viable. The highest average of pollen viability was observed in *R. lineatus*, followed by *R. fraxinifolius* (98 %) and the lowest pollen viability showed by *R. alceifolius* (59,68 %). The non-viable pollen grains were much smaller than those that viable and were often considerably deformed, as seen in Figure 1.b. The standard pollen staining method is using 1% I_2KI solution, which reveals starch content in pollen [19]. Analyzing pollen viability using staining method has various advantages i.e., speedy and applicable to virtually all species, but frequently obtained different results leading to an overestimation of pollen viability [20]. Therefore, another frequently used method, such as in vitro germination methods, is necessary to assess actual values of viable pollen.

TABLE 1. Pollen viability percentage (%) of Rubus spp.

Species of Rubus	Viability (%)±SE(m)
Blackberry	68.77±2.716
R. ellipticus	91.33±2.328
R. fraxinifolius	98.09±1.033
R. rosifolius	93.29±1.967
R. alceifolius	59.68±3.486
R. chrysophyllus	91.99±2.405
R. lineatus	98.11±0.916
R. moluccanus	91.52±3.192
R. pyrifolius	81.53±2.340
Raspberry	84.10±2.039



(a) (b)

FIGURE 1. (a) Viability of pollen *Rubus* spp. (b) Pollen grains of *Rubus alceifolius* in 1% I_2KI solutions.
NV: non-viable pollen, V: viable pollen

The pollen grains that staining intensely when observed under the microscopic field was counted as viable, whereas poorly stained were considered as non-viable. The highest average of pollen viability was observed in *R.lineatus* followed and *R. fraxinifolius* that about 98 % and the lowest pollen viability showed by *R. alceifolius* (59,68 %). As seen in Figure 1.b, the non-viable pollen grains were much smaller than those that viable pollen and were often considerably deformed. The common pollen staining method is using I_2KI 1% solution which reveals starch content in pollen [19]. Analyzing pollen viability using staining method has various advantages i.e. speedy and applicable to virtually all species, but frequently obtained different results leading to an overestimation of pollen viability [20]. Therefore, another frequently used method such as *in vitro* germination methods is necessary to assess actual values of viable pollen.

Pollen Germination and Pollen Tube Lengths

The germinated pollen grains were observed at 1, 3, 6, and 24 hours in BK media. Data in Table 2 and Figure 2 showed that *R. linneatus* has the fastest pollen germination growth among the ten species observed, with the average percentage was 59.92 % at 1 hour of germination, and still stable as the highest ones up to 24 hours with 69.87 % of pollen germinated. On the other side, Blackberry indicated slow and delayed germination at different times of incubation. The percentage of pollen germinated only 1.7% at 1 hour, but then increases considerably after 24 hours, with 26.13% of pollen germinated.

The pollen germination process can take from a few minutes to several hours/days and has a different rate between species, depending on the species and the given treatments. In the research of Pawar et al. [7] on the *R. ellipticus*, showed the highest pollen germination was observed after 48 hours (36,66 %) when grown in solution with 25% sucrose and 0,4% boric acid. When the pollen germinated in solution with 10 % sucrose and 0,1% boric acid, which almost similar to the media used in our research, the percentage of germination at 12, 24, and 48 hours was 7 %, 11,66%, and 14% respectively. The results at 24 hours similar to that observed in table 2, in which at 24 hours, only 13.57% of pollen germinated. Based on both experiments, it showed that the media could affect germination. Hiregoudar et al. [21] reported pollen of *R. paniculatus,* showed delayed germination at 24 hours. The same pattern was observed in blackberry in this research. Moreover, the observations on eight apricot cultivars [22] also showed that viability and germination pollen rates differed among species/cultivar.

In vitro germination was a standard method to assess pollen viability, which is provided more reliable data than the staining method. However, *in vitro* germination conditions are not optimal for the pollen tube formation in many species, and therefore germination percentages will usually be lower than actual pollen viability rates [20].

TABLE 2. Pollen germination precentage of *Rubus* spp. after different time of incubation.

Species of Rubus	pollen germination (%)±SE(m) (1hour)	pollen germination (%)±SE(m) (3hour)	pollen germination (%)±SE(m) (6hour)	pollen germination (%)±SE(m) (24hour)
Blackberry	1.70±1.191	4.78±2.162	6.85±2.724	26.13±5.849
R. ellipticus	20.56±1.237	15.46±4.957	14.76±3.100	13.57±2.509
R. fraxinifolius	14.40±3.630	39.053±4.646	26.66±2.049	36.92±19.942
R. rosifolius	10.35±1.651	39.07±10.921	66.54±4.469	54.18±8.385
R. alceifolius	9.47±3.351	7.73±2.081	16.66±1.134	18.76±2.812
R. chrysophylus	20.83±14.812	67.99±16.896	75.84±11.528	51.18±1.410
R. lineatus	59.92±15.78	63.17±17.88	79.84±10.081	69.87±8.337
R. moluccannus	33.16±8.038	30.87±4.520	22.63±3.292	27.12±4.974
R. pyrifolius	8.85±4.618	12.94±2.893	14.75±7.737	28.26±6.772
Raspberry	16.39±5.307	30.91±6.089	28.87±7.214	48.29±10.071

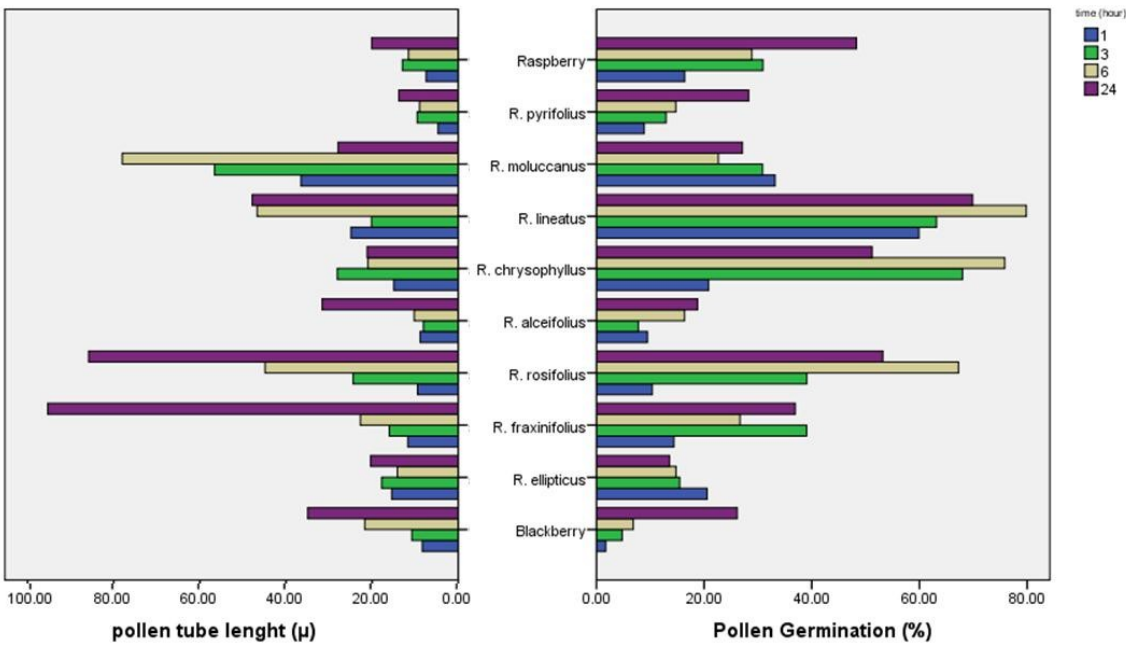


FIGURE.2. Pollen tube length (**µ**) of *Rubus* spp. and pollen germination percentage (%) of *Rubus* spp

TABLE 3. Pollen tube length of *Rubus* spp. after different time of incubation

Species of Rubus	pollen tube length (µ)±SE(m) (1hour)	pollen tube length (µ)±SE(m) (3hour)	pollen tube length (µ)±SE(m) (6hour)	pollen tube length (µ)±SE(m) (24hour)
Blackberry	9.22±1.008	11.35±0.866	24.68±2.165	35.339±3.784
R. ellipticus	15.08±1.436	16.19±1.802	14.24±1.203	20.47±1.723
R. fraxinifolius	9.66±0.601	18.39±2.263	24.19±3.687	108.56±13.509
R. rosifolius	9.49±2.02	24.39±2.731	37.23±6.083	88.12±17.394
R. alceifolius	8.97±0.654	8.09±0.015	10.22±1.473	30.203±6.888
R. chrysophylus	20.06±3.884	28.08±5.075	20.97±4.307	20.14±4.076
R. lineatus	26.69±2.884	20.13±1.976	46.71±7.158	47.83±6.382
R. moluccannus	27.15±5.582	36.03±5.817	44.92±8.349	46.45±11.001
R. pyrifolius	4.69±0.813	11.67±1.049	10.71±1.193	14.18±2.478
Raspberry	7.22±0.457	12.09±1.329	13.02±1.679	23.03±4.881

The data of pollen tube length was observed after different times of pollen incubation, and the results are presented in Figure 2 and Table 3. The longest pollen tube (108.56 µ) was recorded on *R. fraxinifolius* after 24 hours germinated, and the shortest pollen tube (14.18 µ) was recorded *R. pyrifolius*. The longevity of pollen *R. fraxinifolius* and *R rosifolius* rise significantly after 24 hours germinated with pollen tube length 108.56 µ and 88.12 µ, respectively. On the other hand, *R chrysophyllus* has stagnant growth of pollen tube for 24 hours with about 20 µ length.

TABLE 4. Spearman rank correlation cofficients for average of pollen germination, pollen viability, and pollen tube lenght *Rubus* spp.

Correlation Coefficient	Pollen germination	Pollen viability	Pollen tube
Pollen germination	1	0.537**	0.587**
Pollen viability		1	0.443**
Pollen tube			1

** Correlation is significant at the 0.01 level

In order to know the correlation between pollen viability, germination, and pollen tube, the Spearman rank correlation was done. Based on Table 4, it showed that pollen germination of *Rubus* spp. was positively correlated with pollen viability and tube length when tested with the Spearman rank correlation coefficient.

Pollen size

The measurement of pollen size was presented in Table 5 as an average pollen area. The largest viable and non-viable pollen size was showed by *R. alceifolius* with 1306.03 µ and 541.84 respectively. The smallest size of viable pollen was showed by *R. fraxinifolius* with 426.82 µ pollen area, and the smallest of non-viable pollen area was Raspberry with 187.32 µ. Pollen grains of *Rubus* in mass pollen grain looked golden yellow in color and have a different shape in dry and watery conditions (Figure 3) [21]. The shapes of pollen grains in dry conditions were oblong and elliptic, while in wet conditions, pollen appeared to be a round shape. The viable pollen tends to have a round shape due to nutrients absorb more water than non-viable pollen. In this data, pollen size counted after sowed at 1 % I_2KI solution.

TABLE 5. viable and non-viable pollen size of *Rubus* spp.

Species of ***Rubus***	viable pollen area (µ)±SE(m)	non-viable pollen area (µ)±SE(m)
Blackberry	852.63±10.515	317.15±5.260
R. ellipticus	433.45±6.032	201.69±5.305
R. fraxinifolius	426.82±6.414	221.51±8.605
R. rosifolius	431.91±7.058	254.84±9.644
R. alceifolius	1306.03±32.401	541.84±13.852
R. chrysophyllus	927.31±21.805	407.62±43.439
R. lineatus	1178.34±22.029	413.93±21.370
R. moluccanus	727.78±15.978	362.12±13.307
R. pyrifolius	800.10±20.324	409.43±11.918
Raspberry	569.45±7.989	187.32±3.812

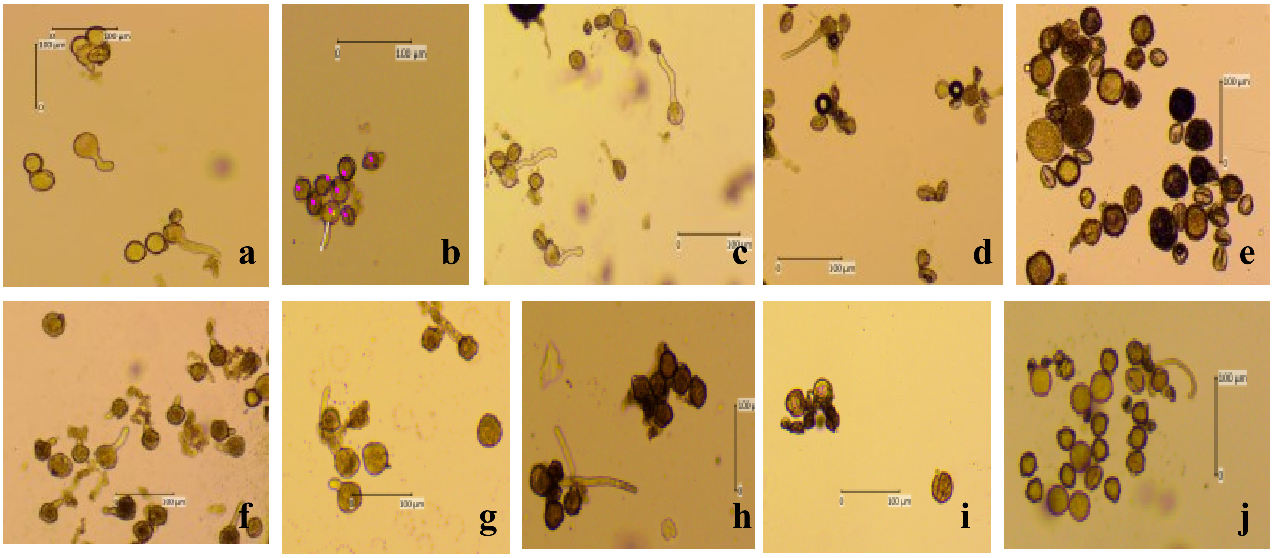


FIGURE 3. Comparison pollen grains of *Rubus* spp (a) Blackberry (b) *R. ellipticus*, (c) *R. fraxinifolius* (d) *R. rosifolius*, (e) *R. alceifolius*, (f) *R. chrysophyllus*, (g) *R. lineatus*, (h) *R. moluccanus*, (i) *R. pyrifolius*, (j) Raspberry

CONCLUSSION

The viability, germination, and tube length of *Rubus* spp. pollen varied according to species and methods. The viability of pollen *Rubus* ranged from 59.68 % to 98.12%. However, *R. linneatus* and *R. fraxinifolius* were generally higher than the other species in the parameters. There was a positive correlation between germination, viability, and pollen tube length.

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