Pollination and hybridisation systems in genus *Ribes* L. (Grossulariaceae)

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**Abstract.** Currants are important berry crops cultivated for medicinal purposes. Most currant cultivars are crossings between *Ribes nigrum* varieties, while many *Ribes* species are not used in hybridisation. Recent research on Ribes crossbreeding is scarce. Hybridisation of wild species can improve reproductive success and the taste of fruit. However, hybrids of certain currant species cannot produce fully formed berries. Crossbreeding of currant species was performed in Northwest Russia (Saint Petersburg Botanical Garden). The research included both introduced (*R. sachalinense*, *R. sanguineum*, *R. rubrum*, *R. hispidulum*, *R. mandschuricum*, *R. spicatum*, *R. latifolium*) and native species (*R. aureum*, *R. alpinum*, *R. nigrum*). The research aimed to identify the hybridisation system, pollination mechanisms and hybrids with the highest percentage of fruit and seed set. Pollen fertility and viability were assessed. Pollen is fertile (acetocarmin staining: 80.3% for *R. alpinum* and 96.0% for *R. mandschuricum*); germination is active on nutrient medium and in situ, especially in *R. sachalinense*, *R. aureum* and *R. spicatum* (54.2–41.8%). Pollen germination can be irregular. Flowers were castrated before pollination. Optimal hybrids with a high percentage of fruit set were identified: *R. alpinum* × *R. mandschuricum*, *R. alpinum* × *R. aureum*, *R. sachalinense* × *R. sanguineum*, *R. rubrum*, *R. hispidulum*.

1. **Introduction**

Currants are important berry crops; their fruit, stems and leaves have long been used for medicinal purposes. They contain antioxidants, minerals as well as the largest number of vitamins among berry plants. Currants are leaders among other berry crops in the Russian State Register of Protected Breeding Achievements. The genus *Ribes* L. comprises 100–150 species distributed throughout the Northern temperate zones of both hemispheres, the mountains of Central America and North Africa [1]. The species are deciduous, rarely evergreen shrubs with smooth or prickly stems and regular lobed leaves. Flowers are in racemes, sometimes in clusters or solitary. Plants are dioecious or monoecious.

Despite their diversity, most of the existing currant cultivars were produced by crossing cultivars of the *Ribes nigrum* species [2–10]. Approximately 1200 varieties of blackcurrant cultivated in Russia nowadays were mainly developed from 10 species of the genus *Ribes*. Few studies are devoted to developing cultivars of red currant—*Ribes rubrum*. Golyaeva [11] provides a list of the red currant cultivars included in the State Register of Breeding Achievements and approved in Russia. Most *Ribes* cultivars are developed from two or three species [12–14]. The aim of the currant selective breeding is to improve plant adaptation [15], winter frost resistance [16] and fruit attributes [5, 17–20].
In practice, many wild species of *Ribes* are not used in selective breeding. However, using wild species in selective breeding can improve parameters that influence reproductive success and the taste of fruit. However, not all species of *Ribes* can be crossed to produce hybrids with fully formed berries, especially outside native distributional range. Available data on crossings between the species of *Ribes* is outdated and fragmentary. Hybridisation of introduced species of *Ribes* with native species remains poorly studied; available research mainly describes the biology and morphometrics of the introduced plants.

For successful breeding process, it is important to use viable pollen capable of fertilisation. Researchers define pollen viability for the species of the genus *Ribes* as high—the vast majority of grains are mature and characterised by high fertilising capacity [21]. Using fertile pollen in hybridisation of the *Ribes* species can raise the quality of selective breeding to the next level [22].

The above-mentioned issues formed the basis for a study in which crossings of different species of currants were carried out in Saint Petersburg (Saint Petersburg Botanical Garden, Komarov Botanical Institute of the Russian Academy of Sciences [Botanical Garden]). The study aimed to identify the hybridisation system and pollination mechanisms in the species of *Ribes* as well as to cross various species of *Ribes* in order to identify hybrids that demonstrate the highest percentage of fruit and seed sets. To achieve this aim, a laboratory assessment of pollen grain fertility and viability was performed; pollen grain germination on the stigma of the gynoecium and fruit set potential of different hybrids were studied.

2. Material and methods

Species of *Ribes* analysed in this research include both introduced species (*R. sachalinense, R. sanguineum, R. rubrum, R. hispidulum, R. mandshuricum, R. spicatum, R. latifolium*) and native species (*R. aureum, R. alpinum, R. nigrum*) from the Botanical Garden. Characteristics of the species are described in the article by Firsov and Yandovka [1].

The flowers were castrated for hybridisation. After pollination, the stems were covered with gauze bags. Fresh pollen (no more than 10 days old) was used for the experiment. Pollen fertility was evaluated by acetocarmine staining and germination on the artificial nutrient medium [23]. The pollen tube passage in the style of the gynoecium was studied by preparing anatomical sections which were later analysed with the Zeiss EVO-40 electronic microscope at the Herzen University Center for Collective Use of Atomic Force and Electron Microscopy. Productivity (fruit formation) indicators were determined by counting the number of generative diaspores—flowers and fruit. The number of registered stems is no fewer than 10.

3. Results

Pollen grains of the *Ribes* species are spheroidal and tricolpate. Research literature contains information about pollen morphology of Grossulariaceae species [24–28 et al.]. Gavrilova and Tikhonova [12] note that pollen of Grossulariaceae is pororate, except for three species from the section Heritiera (*R. sachalinense*, etc.) with colporate pollen grains.

In the studied *Ribes* species, deformed pollen is found along with normally shaped pollen (figure 1). Small and medium-sized deformed pollen grains are characterised by compressed cell contents. Thus, their shape is oblate or elongated instead of round. Such pollen grains often cannot be stained with acetocarmine; therefore, they are morphologically immature. Too large pollen grains of *Ribes* are predominantly deformed; their shape is oval instead of round. Such pollen grains often remain unstained and cannot germinate on the artificial nutrient medium.
Figure 1. *R. latifolium* pollen grains (acetocarmine staining). *dp* stands for deformed pollen; *sp* stands for acetocarmine-stained pollen grains; *up* stands for unstained pollen.

As can be seen from table 1, *R. alpinum* (10.0%) and *R. sachalinense* (3.5%) have the most deformed pollen grains. No pollen shape deformation was observed in *R. latifolium*. Normally formed (fertile) pollen grains are stained with acetocarmine. As can be seen from table 1, the studied species show a high percentage of pollen staining - 80.3% for *R. alpinum* and 96.0% for *R. mandschuricum*.

Table 1. Pollen grain fertility of *Ribes* species determined by acetocarmine staining.

| Species             | Total number of pollen grains analysed | Pollen grains deformed | Pollen grains stained | Pollen grains length | Large | Medium | Small |
|---------------------|----------------------------------------|------------------------|-----------------------|----------------------|-------|--------|-------|
|                     |                                        | number                 | %                     | number               | %±m  | %      | %     |
| *R. sachalinense*   | 254                                    | 9                      | 3.5                   | 236                  | 92.9 ± 1.6 | 8.9 | 84.8 | 6.2  |
| *R. sanguineum*     | 314                                    | 1                      | 0.3                   | 280                  | 89.2 ± 1.7 | 1.9 | 92.7 | 5.4  |
| *R. rubrum*         | 260                                    | 3                      | 1.2                   | 234                  | 90.0 ± 0.6 | 4.3 | 79.9 | 15.8 |
| *R. hispidulum*     | 261                                    | 3                      | 1.1                   | 249                  | 95.4 ± 1.3 | 11.5 | 78.2 | 10.3 |
| *R. aureum*         | 606                                    | 3                      | 0.5                   | 529                  | 87.3 ± 1.3 | 2.0 | 94.6 | 3.4  |
| *R. spicatum*       | 549                                    | 14                     | 2.6                   | 480                  | 87.4 ± 1.4 | 3.3 | 91.3 | 5.4  |
| *R. mandschuricum*  | 427                                    | 4                      | 0.9                   | 410                  | 96.0 ± 0.9 | 2.6 | 94.4 | 3.0  |
| *R. alpinum*        | 396                                    | 36                     | 10.0                  | 318                  | 80.3 ± 2.0 | 4.5 | 81.8 | 13.6 |
| *R. nigrom*         | 286                                    | 6                      | 2.1                   | 268                  | 93.7 ± 1.4 | 6.3 | 86.7 | 7.0  |
| *R. latifolium*     | 425                                    | 0                      | 0                     | 385                  | 90.6 ± 1.4 | 0.2 | 96.7 | 3.3  |

Pollen grains were divided into three groups based on their size: (1) small pollen (less than 20 microns); (2) large (more than 45 microns); and (3) medium (21–44 microns). The ratio of small, large, and medium pollen grains varies among *Ribes* species (see table 1). It is known that medium-sized pollen grains germinate most actively on the stigma of the gynoeicum. The largest number of medium pollen grains is observed in *R. latifolium* (96.7%), *R. mandschuricum* (94.4%) and *R. aureum* (94.6%). During germination on the artificial nutrient medium, only a few small pollen grains begin and then quickly stop growing. *R. rubrum* (15.8%) and *R. alpinum* (13.6%) have the largest number of small pollen grains.

Despite the fact that the pollen of the studied plants is mostly mature (confirmed by acetocarmine staining), in a number of species it does not germinate well on the artificial nutrient medium (table 2). Pollen grains of *R. aureum* (52.9%) and *R. sachalinense* (54.2%) demonstrate the highest rate of successful germination. Total number of pollen grains that grew into long and medium-length fertile pollen tubes is the highest for *R. aureum* (71.1%) and *R. nigrum* (57.9%).
| Species              | Total number of pollen grains analysed | Number of grains grown into pollen tubes total, % ± m | long, % | medium, % | short, % |
|---------------------|---------------------------------------|------------------------------------------------------|--------|-----------|---------|
| Ribes sachalinense  | 441                                   | 54.2 ± 2.3                                          | 14.2   | 28.5      | 57.0    |
| R. sanguineum       | 311                                   | 31.8 ± 2.6                                          | 8.1    | 29.3      | 62.6    |
| R. rubrum           | 286                                   | 33.9 ± 2.7                                          | 17.5   | 29.9      | 52.6    |
| R. hispidulum       | 327                                   | 22.9 ± 2.3                                          | 0      | 25.3      | 74.7    |
| R. aureum           | 340                                   | 52.9 ± 2.7                                          | 40.0   | 31.1      | 28.9    |
| R. spicatum         | 402                                   | 41.8 ± 2.4                                          | 7.1    | 48.2      | 43.5    |
| R. mandschuricum    | 225                                   | 14.2 ± 2.3                                          | 12.5   | 25.0      | 62.5    |
| R. alpinum          | 329                                   | 17.0 ± 2.1                                          | 8.9    | 26.8      | 64.3    |
| R. nigrum           | 292                                   | 26.0 ± 2.5                                          | 21.1   | 36.8      | 42.1    |
| R. latifolium       | 320                                   | 20.3 ± 2.2                                          | 0      | 46.2      | 53.8    |

All species demonstrate both normal pollen germination (straight pollen tubes; figure 2, a) and irregular formation of pollen tubes. In the latter case pollen tubes may change their growth direction (figure 2, b, c) or stop at the beginning of growth, may twist or develop from one pollen grain, etc.

**Figure 2.** Pollen germination of *Ribes* on the artificial nutrient medium. a - *R. nigrum*; b - *R. spicatum*; c - *R. latifolium*.

When pollen gets on the stigma of the gynoecium (*in situ*), its active germination begins. According to A.S. Afanasieva [27], after the flower blossoms, it takes pollen tubes of the *Ribes* plants from 96 to 120 hours to pass through the gynoecium and enter the ovary. A.F. Popova [28] notes that two days after gametes fuse in the ovule. Our research results question the credibility of the two above statements. The Northwestern District of Russia, where the study was conducted, is characterised by sharp air temperature changes, which can occur within one day. At a relatively high air temperature, it takes the pollen tube 3–5 days on average to reach the ovule, which is consistent with the data provided by A.A. Radionenko [29]. When the temperature decreases, the pollen tube can grow inside the pistil tissues for up to 10–12 days, stop growing altogether, or stop for some time (up to 3 days) and then degenerate. Mature embryo sacs ready for fertilisation are observed in the ovules on the first day of flowering. According to V.Yu. Mandrik [30], the monosporic embryo sac of *Ribes* contains eight nuclei and, in a mature state, consists of an egg apparatus in the form of two synergids and an egg, a central cell and an antipode.

The surface of the stigma has numerous grooves, ensuring firm pollen adhesion (figure 3, a). When a pollen grain lands on the stigma, the generative cell divides into two sperm cells that enter the pollen tube produced from the vegetative cell. Even at a low positive air temperature, almost all pollen grains located on the stigma start growing (figure 3, a). Some of the pollen grains may start growing 2–3 days...
after they land on the stigma. Many pollen grains start growing and then stop—they produce short pollen tubes and then degenerate. Some pollen tubes stop growing at different lengths while passing through the stylar canal, or deform after growing a little (figure 3, b) and then degenerate. Cases where pollen grew into the stylar canal through the wall of the style and not through the stigma were observed in all the analysed species (figure 3, c). Once in the ovary (figure 3, d), the pollen tube continues to grow actively; it can bend, curl and fold multiple times (figure 3, f) until it reaches the micropyle of the ovule. Four hemi-orthotropous ovules develop on the walls of the ovary; according to I.I. Shamrov [31], they are located at 6–10 levels (figure 3, e).

Interspecific hybridisation of the studied plants is not always successful. At the same time, as noted by Melekhina and Yakobson [32], intraspecific hybrids of different R. nigrum forms are characterised by rapid germination of the pollen tube in the gynoecium (the pollen tube grows into the ovary 24 hours after pollination; double fertilisation occurs 54 hours later) and a high potential for the fruit set development. The results of the research show that hybrids between certain species do not produce fruit because of their incompatibility. The absence of fruit set was also observed due to different flowering periods of the crossed plants. Pollination of newly opened flowers with pollen collected earlier than 8–10 days before the experiment was not successful. In most unsuccessful crosses, one of the reasons for the lack of fruit set was disorientation of pollen tubes in the ovary. Once in the ovary, they not only grow randomly and bend (figure 3, f) but can also pour their contents into the ovary before reaching the micropyle.

Table 3 shows the results of successful crosses between the studied species. A high percentage of fruit set is found in the following combinations: R. alpinum x R. mandshuricum (95.1%), R. alpinum x R. aureum (94.9%), R. mandshuricum x R. aureum (41.7%), R. nigrum x R. sanguineum (39.3%), R. nigrum x R. aureum (29.2%). Hybrids between R. sachalinense and the species noted in table 3 also demonstrated good results. It should be noted that in the hybrid R. alpinum x R. aureum, the size of the developed fruit was 1.5–1.7 times larger than that of the original species. This might be due to heterosis. The obtained result should be checked in further studies. Some studies provide data that no sets of seeds and fruit are formed when R. aureum is used as a female parent and R. alpinum is used as a male parent; however, crossings similar to ours result in fruit set [12].
Figure 3. Ribes pollen growth in the pistil. \(a\) – a fragment of the stigma; \(b\) – deformation of a pollen tube in the stylar canal; \(c\) – germination of a pollen tube through the wall of the style; \(d\) – germination of a pollen tube in the stylar canal; \(e, f\) – the ovary in longitudinal section; \(o\) – the ovary; \(p\) – a fragment of the pistil; \(pt\) – a pollen tube; \(ov\) – the ovule; \(ow\) – the ovary wall; \(c\) – the stylar canal, \(po\) – pollen.

Table 3. Hybrid combinations of Ribes species and their fruit set.

| Hybrid combinations    | Number of pollinated flowers | Fruit set number | Fruit set % ± m |
|------------------------|-----------------------------|-------------------|-----------------|
| Ribes spicatum         | 296                         | 2                 | 0.7 ± 0.8       |
| R. aureum              | R. mandshuricum             | 276               | 25.4 ± 2.6      |
|                        | R. mandshuricum             | 669               | 95.1 ± 0.8      |
|                        | R. aureum                   | 432               | 94.9 ± 1.1      |
| R. nigrum              | R. mandshuricum             | 350               | 14.0 ± 1.8      |
|                        | R. aureum                   | 360               | 29.2 ± 2.4      |
|                        | R. spicatum                 | 411               | 2.2 ± 0.7       |
|                        | R. alpinum                  | 532               | 3.0 ± 0.7       |
|                        | R. sanguineum               | 488               | 39.3 ± 2.2      |
| R. aureum              | R. mandshuricum             | 480               | 41.7 ± 2.2      |
Notes. The table shows the hybrids of *Ribes* species that demonstrated the highest percentage of fruit set. The results of crosses that showed a low percentage of fruit set or no fruit set at all were not included in the table.

### 4. Conclusion

The pollen of the studied *Ribes* species is fertile (stained with acetocarmine and actively growing on the artificial nutrient medium). Pollen grains with deformed morphological structure were observed along with the regular ones. A wide range of pollen size is observed: the ratio of large, medium and small pollen grains varies among species. Since medium-sized pollen demonstrates the most active growth on the stigma of the pistil, its quantity is of special importance. The largest number of medium pollen grains is observed in *R. latifolium*, *R. mandschuricum* and *R. aureum*. The percentage of successful pollen germination on the artificial nutrient medium is generally low, while pollen grains of *R. aureum* and *R. sachalinense* grow better than the others. The total number of pollen grains that grew into long and medium-length pollen tubes (with the highest fertilising capacity) is the highest for *R. aureum* and *R. nigrum*. Pollen germination of *Ribes* species is active on the stigma of the gynoecium (*in situ*), but may be irregular.

Not all species of *Ribes* can cross between each other due to incompatibility, different flowering periods and disorientation of pollen tubes in the ovary. Combinations of *R. alpinum* x *R. mandschuricum*, *R. alpinum* x *R. aureum*, *R. sachalinense* x *R. sanguineum*, *R. rubrum*, *R. hispidulum* demonstrated a high percentage of fruit set, therefore, they can be recommended for breeding.

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