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IN VITRO GERMINATION OF POLLEN OF SOME *LONICERA* L. SPECIES

Abstract

The morphological structure of flowers of *Lonicera* L. species, the study of their biology, and observations show that the studied species are mainly cross-pollinated plants. Bees, ants, and other insects visit flowers for nectar, and thus they are considered one of the main pollinators of the plant. Pollen viability is an important indicator in the study of the generative development of plants. Pollen viability can be objectively evaluated using the method of germination in an artificial food medium. There is little literature data on pollen germination of *Lonicera* L. species, based on which, the fertility and sterility of pollen, and the effect of concentrations of different substances on pollen germination were studied.

Keywords: *ornamental plants, introduction, Lonicera L., pollen, pollen tube*

Introduction

Lonicera L. species are mainly distributed in the temperate and subtropical regions of the northern hemisphere. Most species grow in the thickets of broadleaf, coniferous, mixed, and montane forests of Eurasia and North America, some species are found in the tropical forests of Southeast Asia. 200 species of genus *Lonicera* L. are known in the world flora. About 140 species are cultivated in dendrological collections of botanical institutions of different countries. Representatives of this family are mainly shrubs and lianas, rarely grasses. The leaves are mostly opposite, evergreen, semi-evergreen or deciduous. These plants are distinguished by their characteristic tubular, bell-shaped flowers. A flower is a complex reproductive organ that carries out the reproduction processes of angiosperms. According to most authors, a flower is a specialized, growth-limited, non-branching organ that ensures the realization of the sexual process resulting in the formation of spores and gametes, the formation of seeds and fruits. The main parts of the flower are the receptacle, petal, sepal, stamens, and pistil (Məmmədov, 2011: 49).

The studied plants are mainly cross-pollinated plants, sometimes self-pollination also occurs. The pollen cells of the stamens vary greatly in size, color, and shape. Inside it is the solid cytoplasm and it contains fat, starch, sugar, and other similar substances. While still in the pollen nest, during germination, the pollen cell nucleus divides into 2 cells: a vegetative and a generative cell. During germination, the vegetative cell produces the pollen tube, and the generative cell produces 2 sperm (İbrahimov, 2004: 187).

In a natural case, the dust grain falls into the pistil's mouth to germinate, and it contains nutrients and biologically active substances for normal germination. This substance attaches the pollen grain to the mouth of the pistil and provides nutrients for its germination (Hümbətov, 2017: 567).

The biomorphological characteristics of the studied species of *Lonicera* L. are shown below (Məmmədov, 2015: 291).

Lonicera Caucasica Pall.

It is a low shrub with a height of up to 3 m, an upright trunk, and branches covered with gray bark. Leaves are simple, entire, glabrous, narrowed towards the base, up to 8 cm long. The flowers

are located in pairs or bunches in the axils of the leaves, they are fragrant and nectar. Fruits are black, spherical, paired in leaf axils.

Lonicera fragrantissima Lindl. & Paxton

The height of the plant is 2-3 m, it is an upright bush with many branches. The leaves are dark green, up to 8 cm long and 3.5 cm wide. The flowers are yellowish-white in color and have a nice fragrance. Each pair of flowers is about 1 cm long. The flowers attract attention before the plant is fully leafed. This plant is considered "The harbinger of spring".

Lonicera japonica Thunb.

The flowers are white, and yellow in color and have a sweet vanilla scent. The leaves are ovate or ovate-oblong in shape. The base of the leaf blade is heart-shaped. Leaf length is 3-8 cm. Young leaves are hairy on both sides, older leaves are smooth. The fruits are black and shiny.

Lonicera korolkowii Stapf.

It is a 3 m tall, soft hairy, branched, decorative, deciduous shrub. The length of the leaves reaches 3 cm, they are oval or elliptic. The flowers are pink and arranged in pairs. The fruits are spherical, bright orange-red, and remain on the branches until autumn.

Lonicera maackii (Rupr) Herd. – Maaka doqquzdonu

The height of the bush is 4m. Dark green leaves are 5-8 cm long, ovate or elliptic. The flowers are white and 2 cm long. The flowers are located in pairs, the white flowers of the plant later turn yellow or pale. The berries are bright red, and balloon-shaped.

Lonicera tatarica L.

It is a shrub with a height of 1.5-2.5 m. The bark of the trunk is grayish and peels off gradually. Its leaves should be 6 cm long and 3 cm wide and ovate. The flower is large, located in pairs in the leaf axil, the petals are pink or dark pink. The fruit is 6-8 mm in diameter, red or yellowish, spherical, ripens in August, and is located on the branches in pairs, free or slightly contiguous.

Results and Discussion.

One of the important parts of the flower is the stamen. This is called androecium (A). Each stamen consists of three parts: a thin stalk below, a pair of sacs above, and the part that connects the pollen sacs to the stalk. The first of these is called the filament, the second is the anthers or pollen, and the third is the dam (İbrahimov, 2004: 184). To study the viability of pollen and pollen germination of 6 species of *Lonicera* L., stamens of flowers with buds ready to bloom were used. Pollen viability was studied by acetocarmine staining method (Retina, 1981: 75). Determination of the fertility and sterility of pollen was carried out using the dye-acetocarmine, in 10 fields of vision for 4 repetitions. (Chelak, 1989: 31).

First, the anthers were removed from the stamens. Then conditions are prepared for the germination of pollen tubes. At this time, a drop of distilled water is poured on the object glass through a pipette. Pouring a drop of pre-prepared solution on it with a pipette and shaking the anthers with the tip of tweezers, pollen is added to the plant and then covered with a covered glass. (Qaziyev, 2017: 195).

Sucrose (30%), and boric acid (0.0005%) concentrations were used to prepare the nutrient medium (Romanyuk, 1986: 53).

The edges of the cover glass are vaselined so that the glass does not move and the liquid does not evaporate. A chamber with humid conditions created for rapid germination of pollen is placed in the thermostat together with the object. The temperature in the thermostat is raised to 20-25⁰ C (Qasimov, 2010: 196). Germination was determined 24 hours after sowing in 5-6 field of view with 100x magnification of the microscope. When the pollen germinates, that is, when the pollen tube is formed, it is necessary to make sure that the edge of the tube corresponds to the ruler of the micrometer. After that, during one day, the growth of the pollen along the ruler is observed and how many mm it grows is determined.

The effects of substances with different concentrations on the germination of pollen of *Lonicera* L. species were analyzed and determined. Pollens were germinated in petri dishes with a thin layer

of water at the bottom at a temperature of 25°C (Golubinski, 1974: 280). Pollen tubes larger than the diameter of germinated pollen are formed.

In the first part of the study, the pollen of *Lonicera L.* species was divided into 2 groups according to their viability:

Group I - pollen with high viability

(*L. fragrantissima*, *L. japonica*, *L. tatarica*, *L. korolkowii*)

Group II - pollen with moderate viability

(*L. caucasica*, *L. Iberica*)

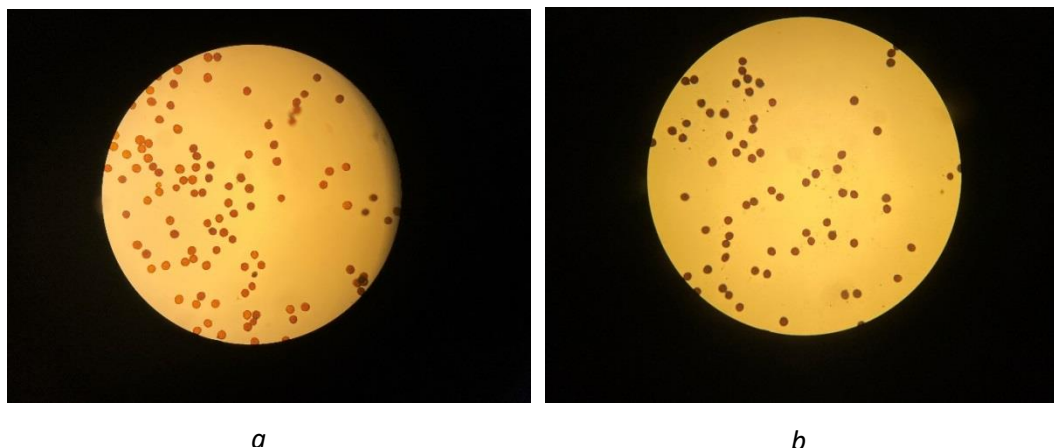


Figure 1. Pollen of *L. iberica* (a) and *L. fragrantissima* (b).

In the second part of the experiment, we studied pollen germination in pure sucrose solutions and the effect of adding agar to the nutrient medium (Romanyuk , 1987: 74). Pollen germination begins with the development of the pollen tube. Usually, germination begins with the outward growth of the pollen cell from the pores on the exine. A nutrient medium is essential for pollen grain growth. The processed protrusion becomes elongated and becomes a pollen tube (Tutayüq, 1967: 242). Germination percentage and pollen tube length were observed in 30% sucrose solution (Table 1). Pollen of *Lonicera L.* species germinates poorly in pure sucrose solutions. Comparing the germination of pollen of the above species in sucrose solutions with the addition of 1% agar-agar, it was found that the percentage of germination is higher than in the previous medium, and the pollen tubes are 2-5 times longer. The addition of boric acid to the composition of the nutrient medium has a stimulating effect on the germination of pollen and the growth of pollen tubes at a concentration of 0.0005% of boric acid. (Table 2).



Figure 2. *L. tatarica*. germination of the species in sucrose solution (with the addition of 1% agar-agar)

Table 1.
Germination results of freshly collected cornflower pollen in sucrose solutions
(with the addition of agar-agar (1%)).

No	Species	Optimal concentration of sucrose (%)	Pollen Germination (%)	Pollen tube length (mm)
1	<i>L. caucasica</i>	25	25	1,63
2	<i>L. fragrantissima.</i>	25	65	3,01
3	<i>L. japonica</i>	30	70	2,15
4	<i>L. korolkowii</i>	25	48	3,07
5	<i>L. maackii</i>	25	45	1,75
6	<i>L. tatarica</i>	25	75	5,15

Table 2.
Effect of boric acid on pollen germination of *Lonicera L.* species.

No	Species	Control		Boric acid		
		pollen germination (%)	pollen tube length (mm)	optimal density (%)	pollen germination (%)	pollen tube length (mm)
1	<i>L. caucasica</i>	23	1,63	0,005	27	1,75
2	<i>L. fragrantissima.</i>	65	3,01	0,005	66	3,51
3	<i>L. japonica</i>	70	2,15	0,005	73	2,75
4	<i>L. korolkowii</i>	49	3,08	0,005	59	3,58
5	<i>L.maackii</i>	45	1,75	0,005	48	1,86
6	<i>L. tatarica</i>	52	5,11	0,005	62	5,57

When the concentration of boric acid exceeds 0.01%, it inhibits the germination of pollen of *Lonicera L.* species. After 4-5 removal of *Lonicera L.* pollen samples from a desiccator for storage and planting at 20°C, changes in humidity have a negative effect on viability.

Conclusion

During determination of fertility and sterility of studied *Lonicera L.* species, the protoplasm of pollens with normal viability is stained dark red, while sterile pollens without viability remain unstained or take on a light yellow color.

An experiment on pollen germination and storage of *Lonicera L.* species showed that better pollen germination was observed in a nutrient medium compared to the germination of freshly collected pollen. To determine their viability, sucrose, agar-agar, and boric acid were used to increase the germination percentage. When stored for a long time, increasing the amount of boric acid and sucrose in the nutrient medium during the germination of plant pollen is appropriate. Determining the optimal concentrations of sucrose showed that the pollen of *Lonicera L.* species germinated better when the concentration of sucrose solution in the nutrient medium was 30%.

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