The research of seed reproduction peculiarities of wild-growing Paeonia L. genus and perspectives of using peony seeds in food-processing industry

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Abstract. Vegetable oil is essential in everyday life of people. Lately, peonies are being widely used in food-processing industry of China – the seeds are processed for oil which is actively used as a food product, containing more than 90% unsaturated fatty acids. The problem of healthy eating is becoming more and more urgent. The task of Russian Federation science and all branches of AIC (agro-industrial complex) is to satisfy the physiological requirements of population in high-quality, biologically complete and safe food products. Production of peony oil in Russia would be of great importance for the economy of our country, as it is ecologically pure and safe food product for human health. In the article we researched the peculiarities of seed propagation and possible reasons for seed dormancy in some Paeonia L. genus. We carried out some bio-chemical tests in order to define the content of peroxidase enzyme, lignin and cellulose in seed peel. We also examined the intensity of photosynthesis and transpiration in 5 species of Paeonia L. genus: P. tenuifolia L., P. suffruticosa Andrews, P. lactiflora Pall., P. anomala L., P. mlokosewitschii Lomak. It was defined that the highest photosynthesis intensity can be observed in P. tenuifolia L. (86.1 mg/dm²h). Then P. mlokosevitschii Lomak. (65.1 mg/dm²h), P. suffruticosa Andrews. (59.3 mg/dm²h), P. anomala L. (32.8 mg/dm²h), P. lactiflora Pall. (2.15 mg/dm²h) follow. The examined species of Paeonia L. genus have high indices of photosynthesis intensity, and are able to adapt to changes in environment. The photosynthesis intensity is known to be one of the most important indices of high seed productivity and yielding capacity. We recommend using peony seeds in food industry for production of quality peony oil.

1. Introduction

Wild-growing peony species have always been among the most popular decorative flower plants not only in Russia, but also all over the world. Many species of Paeonia L. genus are widely used in landscaping, as well as in interior of public buildings and constructions. Peonies are used in cooking and medicine. Dried roots, stems and seeds are considered to be medicinal raw materials.

Paeonia L. genus for a long time belonged to Ranunculaceae, and then Roudolfie separated it into Ranunculaceae family Ranunculaceae together with monotypic genus Glaucidium in 1830. According to data of molecular taxonomy (APG-IV), Paeonia L. genus takes the following position: Angiospermae (Magnoliophyta) division; Dicotyledones (Magnoliopsida) class; Saxifragales order,
and has position next to family of Altingiaceae. In modern flora the genus counts 32 species in 4 subgenus and 6 sections [1, 2].

Species of Paeonia L. genus are among few perennial plants resistant to environmental factors and having wide area of growing. The plants grow primarily in the Northern hemisphere – from East Asia to South Europe; some species can be found in North America and North Africa. In China, peonies are actively used in food industry – the seeds are processed into oil which has antidiabetic activity [3, 4, 5]. In 2011 Chinese Ministry of Health declared peony oil a new source of food.

According to research made, more than 90% of peony oil contains unsaturated fatty acids, including oleic, linoleic and linolenic acids. Taking into consideration that soybean oil is widely used as food oil it contains only 58-69% of unsaturated fatty acids. These indications show that peony seeds oil is more usefull for human health and life than soybean oil [6-10].

For producing peony oil, wild-growing and cultivated species of peonies are used. Decorative species of peonies are not suitable, as most cultivated peonies have terry form of a flower and do not produce seeds. In China, for producing oil from seeds of peony, the following species are used: Paeonia delavayi Franch., Paeonia ostii T.Hong & J.X.Zhang, Paeonia rockii (S.G.Haw & Lauener) T.Hong & J.J.Li, P. suffruticosa Andrews, P. lactiflora Pall.

Research of oil-producing peonies is generally concentrated on cultivating, reproduction, growing, study of resistance of plants, composition of oil and wide use in food production. At present, the experiments are being carried out concerning getting high yields of peony seeds, and high quality of peony oil [11].

The problem of healthy eating is of great importance in our country today [12]. Producing of peony oil in Russia would have great value for economy, as it is ecologically pure and safe food product for human health. In Russia, 7 wild-growing species of Paeonia L. are distinguished. That is why study of ecologic-morphological characteristics and methods of increasing seed productivity of Paeonia L. species growing on the territory of our country is quite essential.

The main problem in growing wild peony species is low seed yielding capacity due to a long period of germination (10-16 months). In this connection, one of the tasks of researchers was study of reasons of seed dormancy of some Paeonia L. species. In our research we carried out bio-chemical analyses aimed at the content of peroxidase enzyme, lignin and cellulose in seed peel.

One of the important indications of high seed productivity and adaptability of living organisms to environmental factors is the photosynthesis intensity. Many scientists showed that the speed of photosynthesis is an important, but complicated factor for defining and prospecting in accumulation of biomass of a crop; as the ability to photosynthesis is controlled by a gene. The yielding capacity may be optimized by means of photosynthesis speed improving. The speed of photosynthesis and transpiration greatly influences the production of oil crops. Photosynthesis has a fundamental importance for accumulation of biomass, as well as for plant productivity increase. The photosynthesis and transpiration intensity in genus Paeonia L. has been studied by many authors [13-19]. In foreign investigations, photosynthesis intensity has been studied in the following papers: Paeonia lactiflora Pall., Paeonia rockii (S.G.Haw & Lauener) T. Hong & J.J. Li, Paeonia delavayi Franch., Paeonia ostii T. Hong & J.X. Zhang, P. suffruticosa Andrews.

In our investigation, in addition to P. suffruticosa Andrews and P. lactiflora Pall., we were the first to examine photosynthesis and transpiration intensity of P. tenuifolia L., P. anomala L., P. mlokosewitschii Lomak.

The aim of this paper was the study of peculiarities in seed reproduction and seed dormancy, the definition of photosynthesis and transpiration intensity in some species of Paeonia L. genus.

2. Materials and methods

The object of research is 5 species of Paeonia L. genus: P. tenuifolia L., P. suffruticosa Andrews, P. lactiflora Pall., P. anomala L., P. mlokosewitschii Lomak., differing from each other in some ecological and morphological characteristics.
P. tenuifolia L. is a herbaceous xeromesophytic plant belonging to subgenus Paeonia, section Tenuifoliae. It grows in South-Eastern Europe, in the Caucuses and the Crimea. The species is adapted to hot and dry summer. It prefers rocky slopes, feather and multy-herbacious vegetation, it can also be found on the edge of oak forest [20].

P. suffruticosa Andrews is a geoaual shrub, belonging to subgenus Moutan, section Moutan. It grows in provinces of Henan, Gansu, Shunys, Anhui, Shensy, Khubei, Tibet, generally in mountainous and subalpical regions, at the height of 2360-4250 meters above the sea level. The plants are adapted to high temperatures and humidity.

P. lactiflora Pall is herbaceous mesophytic plant, belonging to subgenus Albiflora. It grows in South-East of Khabarovsk Territory, Chita and Amur regions, Primorskiy and Altai Territories. It also can be found in China and on the Korean Peninsula. The plant is also spread in forest-steppe, on the edges, open slopes, shrub zones, in the Mongolian oak forests, multi-herbaceous meadows, on the riverbanks and dry rocky slopes with well-drained soil. This species is well-adapted to low temperatures and comparatively high humidity, it is well resistant to shadowing, but can’t stand hot summer and high temperatures.

P. anomal L. is herbaceous mesophytic plant belonging to subgenus Paeonia, section Paeonia. This plant is widely spread in Eastern Europe, China, Mongolia, Eastern and Western Siberia, Altai, Middle Asia. P. anomal L. is frost resistant, it grows on soils rich in humus.

P mlokosewitschii Lomak. is a herbaceous mesophytic plant belonging to subgenus Paeonia, section Flavonia, growing in the Caucuses, in Azerbaijan, Georgia and Dagestan. The plant prefers wet forests and sub-alpic meadows.

The species of Paeonia L. genus under experiment were grown from seeds brought from nature as well as from various botanical gardens of Russia. The peony seedlings were planted in botanical garden of MSU in 2011. These plants have been cultivating in the same environmental conditions. The experiments were carried out in 2016-2018.

In this article the possible reasons of peony seeds dormancy have been studied, the bio-chemical analyses of peroxidase activity in germinated peony seeds has been carried out, the analyses for definition of content of lignin and cellulose in seed peel has been made. Definition of peroxidase activity has been carried out by the method of A.N. Boyarkin [21]. Lignin was examined by the method of Popov (18). Cellulose was defined by the method of Kurshner and Hoffer [22]. The method was in treating the seed peel of peony with solution of zinc chloride in hydrochloric acid.

The experiment on transpiration intensity was carried out by method of L.A. Ivanov [23], which is based on evaluation of leaf water loosing during one hour in relation to a unit of leave area in raw weight (1g). The research of photosynthesis intensity was carried out in August 2017 by the method of photocolorymetry of definition of carbon content in leaves by wet burning in chrome blend [24].

The experimental data received has been statistically processed with the help of modern computer programs.

3. The results and the discussion

The seeds of genus Paeonia L. plants are typical of having the state of simple morphphysiologic epicotyl dormancy. One of the main reasons of slow peony seed growing capacity is undeveloped embryo, and that is adaptive feature preventing seed germination after shedding of seeds. Thus, this adaptation mechanism protects the plant from death in winter, and saves them from being not strong enough at early stages of ontogenetic development. The main advantage of seeds with undeveloped embryo is its development while putting seeds in favorable for germination environment. That is why two-stage stratification with variable temperatures is necessary for germination of wild-growing species of Paeonia L. genus: hypocotyl and the root of embryo are developed at higher temperatures, but epicotyl requires low temperature.

Peony seed has a weakly differentiated cell body, so the embryo development happens in the postembryonic period. That is why prior to shedding of the mother plant the seeds do not germinate immediately. Post development of an embryo is an urgent condition of seed ability to germinate. Time
necessary for completing this process defines the period of morphological dormancy. Post development of an embryo usually takes place in swollen seeds.

Seeds of the researched genus *Paeonia* L. species are rather large (table 1). *P. tenuifolia* L. has shiny, smooth, brown seeds of an ellipsoidal shape, 5.9-7.5 mm long and 3.5-4.9 mm wide. *P. suffruticosa* Andrews has seeds of ellipsoidal shape 7.5-11.5 mm long and 6.5-8 mm wide, of black color with matte gloss. The seed surface is smooth, with depressions on the sides. In the area of seed scar one can see a small scallop. *P. anomala* L. has oval seeds, almost round, shiny-black, glossy, smooth, with brown noticeable oval scar. The seed length is about 6.6 – 8.0 mm, they are 5.1 – 6.0 mm wide. *P. mlokosewitschii* Lomak has black, matte, oval almost round, 6.1 – 7.0 mm long and 4.2 – 6.4 mm wide seeds.

The greater part of a peony seed is taken by endosperm, in the central part of which there is a cavity bridging to embryo cotyledons. The embryo consisting of two leafy cotyledons, hypocotyl and embryo root is situated on a micropilar end of a seed near the scar and is surrounded by lysed cells.

**Table 1. Morphometric characteristic of some *Paeonia* L. genus species**

| Species            | Seed length mm min-max/M (aver.±aver. mist.) | Seed width mm min-max/M (aver.±aver. mist.) | Aver seed weight 1/10 p. of seeds per gr | Seed color               | Seed shape         |
|--------------------|---------------------------------------------|---------------------------------------------|------------------------------------------|--------------------------|-------------------|
| 1                  | 2                                           | 3                                           | 4                                        | 6                        | 7                 |
| *P. tenuifolia*    | 5.9-7.5/6.7±0.2                             | 3.5-4.9/3.8±0.2                             | 0.06/0.7                                | Brown, shiny             | ellipsoidal       |
| *P. suffruticosa*  | 7.5-11.5/9.3±0.1                            | 6.5-8.0/7.5±0.1                             | 0.77/5.1                                | Black, with matte gloss | ellipsoidal       |
| *P. lactiflora*    | 5.5-10/7.5±0.1                              | 4.1-6.8/5.3±0.1                             | 0.12/1.1                                | Dark-brown               | ellipsoidal       |
| *P. anomala*       | 6.6-8.0/7.8±0.2                             | 5.1-6.0/5.4±0.2                             | 0.13/1.3                                | Black, glossy            | Oval, almost round|
| *P. mlokosewitschii*| 6.1-7.9/7.2±0.1                             | 4.2-6.4/5.4±0.1                             | 0.17/1.7                                | Black, matte             | Oval, almost round|

One more reason of long dormancy period is deep physiological dormancy of epicotyl – the point of seedling growth. This is connected with abscisal acid content (AAC) in peony seeds. It can be assumed that an important factor of seed dormancy is accumulation of abscisal acid having inhibitory action on peony germination.

Another reason of slow seed germination capacity is low activity of peroxidase enzyme. Peroxidase belongs to oxidoreductase class, which presents the group of oxidases. It is important in the respiration process of plants, taking part in respiration cycle and catalyzing reactions of transporting hydrogen on air oxygen. In extreme conditions in plants, respiration is activated and peroxidase activity is increased. According to activity of peroxidase it’s possible to estimate the factor influence and plants condition. Oxidation of these or those compounds is carried out by peroxidase with the help of hydrogen peroxide.

In germinated *P. lactiflora* Pallas seeds we found peroxidase enzyme, its activity being 53 %. In non-germinated peony seeds, peroxidase activity is not observed. Thus, peroxidase enzyme can be found only in germinated peony seeds. *P. lactiflora* Pallas has rather high peroxidase activity; consequently, this enzyme has positive influence on further embryo development [25].

Besides, slow peony seeds germination may depend on lignin – natural polymer containing in seed peel, which can reduce seed permeability for water.
Lignin is a compound of almost all land plants and in prevalence among natural high-molecular compounds is after polysaccharides.

Lignin is a compound matter of almost all land plants, and in prevalence among natural high-molecular compounds takes place only after polysaccharides. The lignin content in the wood of needle-leaf trees is 23 %, in broad-leaf trees it is about 38 %. Lignin is found is cell walls and in intercellular space of plants and it connects cellular fibers. Together with hemicelluloses, it defines mechanical strength of stems and branches.

Having defined lignin in germinated and non-germinated seeds of *P. lactiflora* Pallas, and in non-germinated seeds of *P. suffruticosa* Andrews, we received the following results: germinated seeds of *P. lactiflora* Pallas – 52%; non-germinated seeds of *P. lactiflora* Pallas – 52%; non-germinated seeds of *P. suffruticosa* Andrews – 57% (Table 2).

Comparative content of lignin after seed germination does not naturally change due to being the final metabolite of biosynthetic processes, and is non-changed in the process of seeds germination. But its increased content in seed peel of tree-type peonies reduces the speed of their germination due to reduced peel waterproofness due to great amount of this hydrophobic compound [25].

We have also carried out biochemical analyses defining the cellulose content in seed peel of germinated and non-germinated seeds of *P. lactiflora*. Cellulose is a polysaccharide, which is the principal compound of cell walls in all plants. The amount of cellulose is seed peel of *P. lactiflora* Pallas is explained by reduced content of reserved hemicelluloses, which together with cellulose are defined as hardly hydrolyzed polysaccharides.

| Species                   | Lignin, % | Cellulose, % |
|---------------------------|-----------|--------------|
| *P. lactiflora* (germinated seeds) | 52        | 26           |
| *P. lactiflora* (non-germinated seeds) | 52        | 24           |
| *P. suffruticosa* (non-germinated seeds) | 57        | –            |

The important indication of high seed productivity is photosynthesis rate (Figure1). The research of photosynthesis rate was carried out on August 8 2017. It was stated that the highest photosynthesis rate is typical of *P. tenuifolia* L. (86,1 mg/dm² p/h), then follows *P. mlokosevitschii* Lomak. (65,1 mg/dm² p/h), *P. suffruticosa* Andrews. (59, 3 mg/dm² p/h), *P. anomala* L. (32,8 mg/dm² p/h). The minimal indication of photosynthesis rate is typical of *P. lactiflora* Pall. (2,15 mg/dm² p/h). The experimental data received shows that photosynthesis rate in various species of *Paeonia* L. genus goes on in different ways.

On the same day of August 8 2017, the evaluation of transpiration rate was carried out (Figure 2). Being a physiological process, transpiration is closely connected to the process of photosynthesis [26]. Photosynthesis intensity influences transpiration and is responsible for opening and closing of stomata. These species of *Paeonia* L. Genus, according to reducing of transpiration intensity the situation is as follows: *P. tenuifolia* L. (1,5 g/g.p/h). (1 g of water and 1 g of leaves per hour), *P. mlokosevitschii* Lomak. (1,0 g/g.p/h.), *P. lactiflora* Pall. (0,88 g/g.p/h), *P. suffruticosa* Andrews. (0,87 g/g.p/h), *P. anomala* L. (0,80 g/g.p/h).
Figure 1. Photosynthesis rate of genus Paeonia L. species mg/dm² m²/h.

Figure 2. The daily transpiration intensity of genus Paeonia L. species (1 g of water from 1 g of leaves)
4. Conclusion
During long evolutionary development, plants have been interacting with environment, and gradually created morphological and physiological structures having ability of adaptation to environmental changes, generally showing differences in leaves, roots, seeds and other plant characteristics. Analyzing the results obtained, one can note that peony seeds are important means of reproduction and distribution of species *Paeonia* L. genus.

The possible reasons of seed dormancy of *Paeonia* L. genus can be: undeveloped embryo, deep physiological dormancy of epicotyl, caused by abscisal acid presence; low fermentation activity slowing the embryo development inside the seed; high content of lignin in seed peel, which prevents water penetration into the seed and consequently slows down the process of germination. The seed ability while germinating to pass into the state of dormancy is an important adaptive feature, allowing plants to overcome unfavorable environmental conditions and thus ensuring conservation of plant gene pool on the Earth.

Another important physiological plant feature is leaf photosynthesis, which is closely connected with growth and yielding capacity of crops. It was stated that the transpiration rate is connected with the photosynthesis process. The higher the photosynthesis rate is, the higher the transpiration rate is. The results showed that the highest photosynthesis rate can be observed in *P. tenuifolia* L. (86.1 mg/dm² p/h), then follow *P. mlokosevitschii* Lomak. (65.1 mg/dm² p/h), *P. suffruticosa* Andrews. (59.3 mg/dm² p/h), *P. anomala* L. (32.8 mg/dm² p/h), *P. lactiflora* Pall. (2.15 mg/dm² p/h). These species of *Paeonia* L. genus have high photosynthesis rate indices, and are able to adapt to environmental changes. The investigations could be continued in this direction. It could be research of not only ecologic-morphological characteristics and seed reproduction in species of *Paeonia* L. genus, but examination of getting high yielding capacity of peony seeds and high quality peony oil, as well as study of micro-clonal reproduction of wild-growing peony species in culture *in vitro* [27].

It may be concluded that the production of peony oil will find wide application in future in food industry, being a biologically full-fledged and safe food product.

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