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Seed weight and optimal imbibition period for some herbaceous peony (*Paeonia* spp.) species native to Serbia

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Abstract

Herbaceous peonies are plant species with high ornamental and medicinal value. Due to their endangerment, wild peonies are protected by law in Serbia. Possible ways to protect them are propagation and/or cultivation. Peonies can be propagated vegetatively (by rhizome division) or generatively (with seeds). As peony seeds have a double dormancy and their germination is a long-term process, it is important to determine which seed characteristics and pre-treatments have a positive effect on germination. Since peony seed weight is an important seed characteristic, and imbibition period is an effective pre-treatment, the aim of this study was to evaluate the peony species native to Serbia, and to determine seed mass, seed imbibition capacity, the influence of seed weight on imbibition and the influence of habitat on the studied parameters, as the mentioned parameters have hardly been studied for the tested species. The research was conducted in 2021. on three peony species native to Serbia (*Paeonia tenuifolia* L.-fern leaf peony or steppe peony; *Paeonia peregrina* Mill.-Balkan peony or Kosovo peony and *Paeonia daurica* Andrews). According to our one-year research, there was no statistically significant difference in seed weight depending on the natural habitat within the species and also not between *P. peregrina* and *P. daurica*. A significant difference was found in seed weight between steppe peonies and the other two studied species. In agreement with our results, the optimal imbibition time is two to three days for *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica*. The findings can be used as preliminary research for future peony germination studies.

Key words: Paeonia peregrina Mill, *Paeonia tenuifolia* L., *Paeonia daurica* Andrews, seed mass, soaking time

Introduction

Herbaceous peonies have the greatest plant history of any flowering plant genus, with the significant ornamental value of the flower (Sun et al., 2022), as well as the medicinal value of the root (He et al., 2011), petals (Weixing, 2017; Batinić et al., 2022; Čutović et al., 2022) and seeds (Ning et al., 2015). Seeds have also received much attention recently for their ecological (Rudaya et al., 2021) and edible (Qi et al., 2020) value, as well. Herbaceous peonies are native to the Northern Hemisphere and have adapted to a temperate climate with relatively cold winters and dry and hot summers (Marković et al., 2022). Despite their longevity, they are threatened with extinction in their native habitat due to loss of territory and/or excessive collecting. Although peonies are threatened, the genetic variability of peony seeds is an important source of genetic diversity for the maintenance and regeneration of these populations. In addition, peony seeds can be used to establish new populations of peony species in areas where they are locally extinct.

Peony seeds mature slowly, ripening in late summer and dispersing in autumn (Zhang et al., 2018). At the end of development (Figure 1), the seeds of herbaceous peonies are large and dark (Nanjidsuren et al., 2016). The size and weight of the seeds can be influenced by the location, the position of the plant within the site and the time of harvest (Marković et al., 2022). The seed harvest period ranges from July to the end of October, depending on the species, location (altitude, shade, etc.) and year; *P. tenuifolia* matures earlier in July, while *P. peregrina, P. banatica*, *P. mascula* and *P. officinalis* mature later in August (Marković et al., 2022). The best time to collect seeds to achieve the best possible germination is when the follicle opens and the seed coat darkens (Yu et al., 2007; Zhang et al., 2018). Since peony seeds have a double dormancy and their germination is a long-term process, the determination of seed characteristics and pre-treatments that have a positive effect on seed germination is of particular importance for the conservation of the tested endangered species.

Figure 1. The colour changes during the peony seed maturation

The aim of this study was to determine seed weight, seed soaking capacity, the influence of seed weight on imbibition, as well as the influence of native peony habitat on the studied traits, as these parameters have rarely been studied for the mentioned species due to a limited understanding of the physiology of herbaceous peonies.

Materials and Methods

Seed collection

Peony seeds were collected at full maturity from their native habitats and from the Institute's collection. *P. tenuifolia* was collected in July 2021, followed by *P. daurica* and *P. peregrina* in August of the same year. *P. tenuifolia* was harvested from two natural habitats (Deliblato sands, and Gulenovci), *P. daurica* from one natural habitat (Korube) and *P. peregrina* from four natural habitats (Bogovo guvno, Pirot, Golina and Krivi vir) and from the collection of the Institute in Pančevo. The *P. peregrina* populations at the above-mentioned localities, as well as the *P. tenuifolia* populations at Deliblato Sands, each had more than 100 plants; therefore, one third of the seeds of about 30 plants were taken, giving a total number of about 300. The populations of *P. tenuifolia* at locality Gulenovci and of *P. daurica* at Korube are smaller, so about 100 seeds were collected for each locality in order not to endanger the populations. The Ministry of Environmental Protection of the Republic of Serbia has granted permission for wild-collecting (No. 353-01-1467/2021-04, issued on 26. May 2021).

Table 1. Altitude, latitude, and longitude of Serbian herbaceous peony species' habitats (natural and institute collection)

Locality	Altitude (m a.s.l)	Latitude	Longitude
Pancevo-collection	74	44°52'N	20°42’E
Deliblato sands	167	44°57'N	21°03’E
Golina	299	43°46’N	22°19’E
Krivi vir	467	43°49'N	21°46’E
Pirot	666	43°07'N	22°27’E
Gulenovci	840	43°06'N	22°49’E
Bogovo guvno	952	43°33'N	21°46’E
Korube	954	43°30'N	21°42’E

Determination of the dry weight and moisture content of seeds

After seed collection, the dry weight and moisture content of the seeds were determined, followed by measurements of imbibition and seed weight. The measurement of seed moisture content was carried out in a drying oven. Ten seeds from each locality were dried in an oven at 105 °C until no change in weight could be detected. Therefore, measurement was conducted before drying and then after 24, 36 and 48 hours in the oven. The following formula was used to calculate the moisture content of the seeds:

$$\% \text{ Moisture content} = \frac{\text{Weight of fresh seeds} - \text{Weight of dry seeds}}{\text{Weight of fresh seeds}} \text{x}100$$

Seed weight measuring

At the beginning of the experiment, undeveloped seeds were isolated and calculated separately; their percentage ranged between 3% and 6% (Table 1). Normal seeds were divided in triplicate before the mass of each seed per location was weighed on an electronic balance. The mean mass of the seeds per site, as well as the variation from the mean value, were calculated using the collected data. The differences between localities were also analysed. The mass of seeds was recalculated based on the mass of absolutely dry seeds.

Imbibition capacity

To investigate the impact of a seed imbibition period (control, 1 to 7 days) at a temperature of 22 ^{o}C under laboratory conditions, 30 seeds of each investigated species from each locality, as well as 30 seeds from the Institute collection, were examined. To establish the initial weight, the mass of the seeds was measured before soaking and at 24-hour intervals. In three repetitions, the seeds were kept separately at room temperature in distilled water, wiped with dry blotting paper, and weighed to validate the maximum soaking capacity. The imbibition rate was calculated using the following formula:

$$\text{Imbibition rate} = \frac{\text{Imbibed weight} - \text{Initial weight}}{\text{Imbibed weight}} \text{x}100$$

Results and Discussion

Seed moisture

Based on the results of seeds collected in 2021, the moisture content of peony seeds ranged from 8.3 to 14.5%, depending on locality and species. No difference was observed between the replicates. (Table 2). The difference in moisture level was noticeable within the species of *P. peregrina* but was not significant, most likely the crucial factor for the difference was in altitude of the native habitats of mentioned species. There was a significant difference between the species. Although steppe peonies have smaller seeds and grow in drier environments than other tested peony species, their seeds probably have a higher water-holding capacity.

Table 2. Average seed moisture content of tested herbaceous peony species based on locality

Species	Locality	Average moisture content (%)	STDV
P. peregrina	Bogovo guvno	10.24	0.10
	Pirot	9.71	0.25
	Pančevo	9.64	0.22
	Krivi vir	9.37	0.26
	Golina	8.64	0.21
P. tenuifolia	Gulenovci	14.03	0.14
	Deliblato sand	13.85	0.23
P. daurica	Korube	14.54	0.18

Seed weight

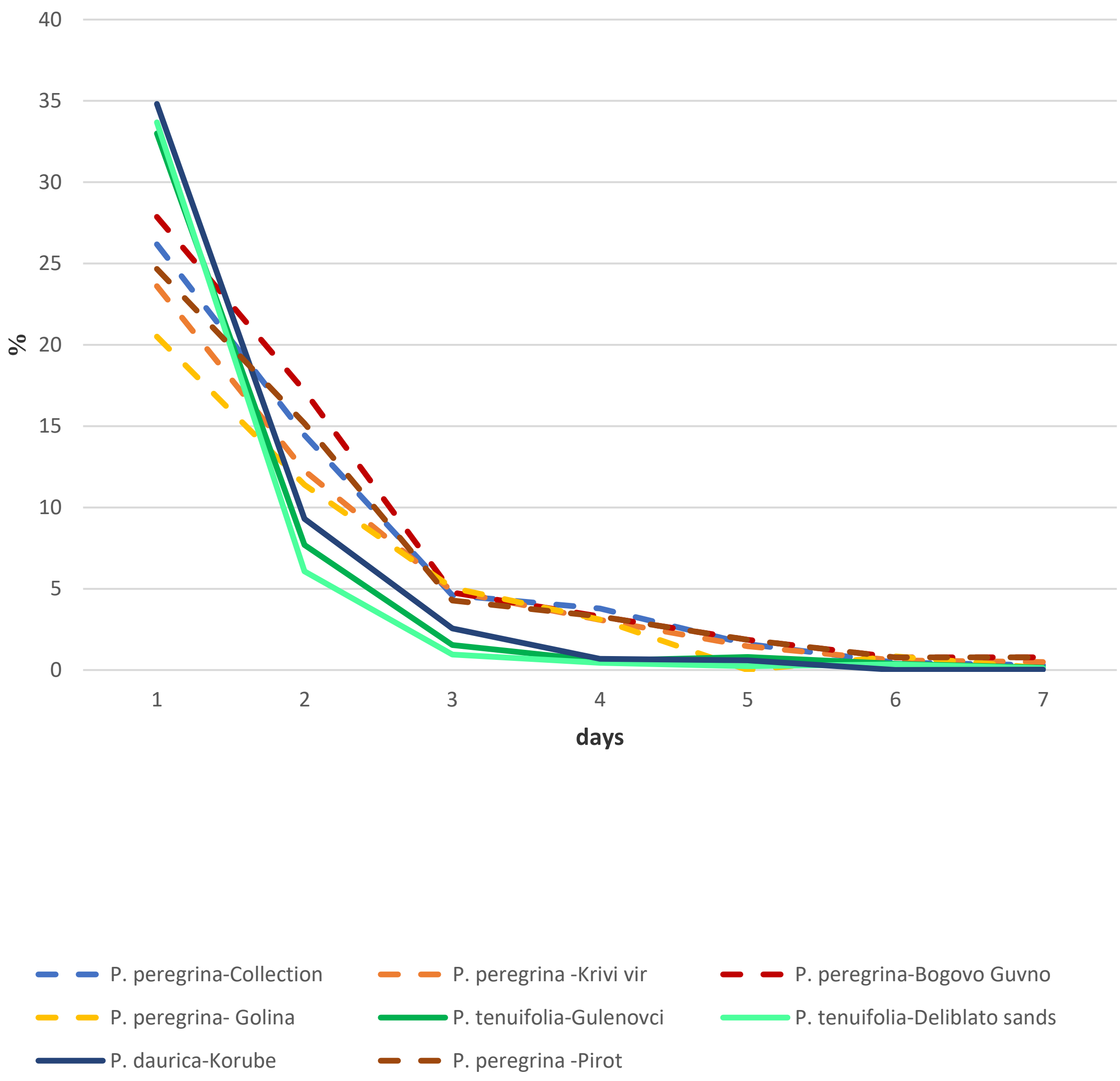
According to research on several plant species (Giles, 1990; Simpson et al., 2021) larger seeds within a species have higher germination energy and bigger seedlings. According to our research on the seeds of herbaceous peonies, there was variety within the species in seed weight according to natural habitat, however the variation is not significant ($p > 0.05$). The variances can be attributed to environmental differences. A significant difference was observed, within a species, between seeds of *P. peregrina* from their natural habitat and seeds harvested in the Institute's collection, which is to be expected, due to better growing conditions and the lack of competition. A significant difference was observed between *P. peregrina* and *P. tenuifolia*, as well as between *P. tenuifolia* and *P. daurica*. Since the seeds of *P. tenuifolia* are the smallest, it was predictable (Hong, 2010). According to our results the species had greater influence on the seed weight than locality. Although the percentage of small seeds was not high for natural habitats, it was probably higher since 2021. year was particularly very dry. *P. tenuifolia* has a higher percentage of stunted seeds, although it tolerates such habitats better, reason could be that in the initial stages of seed development was a particularly dry. Research of Sehgal et al., (2018) indicate that seed productivity might be decreased if early stage of seed maturity is affected by drought.

Table 3. Herbaceous peony seed weight according to species and locality

Species	Locality	Seed weight (g)	Undeveloped seeds (%)
P. peregrina	Pančevo	0.190 ± 0.025	3
	Golina	0.148± 0.023	3
	Krivi vir	0.140 ± 0.024	4.5
	Pirot	0.135 ± 0.047	4
	Bogovo Guvno	0.127 ± 0.025	3
P.tenuifolia	Deliblato Sand	0.051 ± 0.012	5
	Gulenovci	0.061 ± 0.009	5.5
P. daurica	Korube	0.150 ±0.031	3.5

Imbibition capacity

Peony seeds have a hard coat that must be softened and moistened for germination (Yu et al., 2007). The seed coat becomes hydrated during imbibition, allowing water to enter the seed, activating numerous enzymes and initiating metabolic processes that lead to seed germination. Imbibition is the initial stage of seed germination and is essential for plant growth and development. Water soaking has been reported to improve seed germination in both tree and herbaceous peonies (Yu et al., 2007).



Graph 1. Percentage of absorbed water of tested herbaceous peony species from different localities of its initial weight measured every 24 hours for seven days

An herbaceous peony's imbibition period usually lasts differently for different species. According to our findings, the seeds of *P. daurica* and *P. tenuifolia* absorb water more quickly than *P. peregrina*. *P. daurica* seeds absorbed water approximately 35% of their initial weight in the first 24 hours, while *P. tenuifolia* seeds absorbed more than 30%. *P. peregrina* absorbs water more slowly, averaging about 24% in the first 24 hours. *P. peregrina* imbibition capability differs by locality after the first 24 hours, which could be attributed to the initial seed moisture. Dry seeds absorbed the water slower. In the first 24 hours, seeds from the area with lower seed moisture at the beginning of the experiment absorbed water less on average, but there was equalization in the following period. By measuring individual seeds, it was determined that heavier seeds absorb more water, but if it is calculated as a percentage of initial weight, the results showed that the percentage of water uptake was related to the initial weight of the seeds. On the second day, *P. peregrina*, *P. tenuifolia*, and *P. daurica* absorbed 14%, 7%, and 8% of initial weight in water, respectively. On the third day, all species absorbed less than 5% of water. *P. peregrine* on fourth day absorbed 4% water on average, while the other two species absorbed less than 1%. In the following days, all species absorbed less than 1% of water. From the foregoing, it may be proposed two to three days is the optimal time for imbibition of *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica*.

Conclusion

Through the study of herbaceous peony species, an effort was made to ascertain the potential causes of the species' endangerment in their native habitats. As far as seeds are concerned, in herbaceous peonies, over 94% of the seeds are well developed within the species, and no influence of habitat was found; thus, one of the causes for the vulnerability is not the lack of production of quality seed. According to our findings, species had a greater influence on seed weight than locality. According to our research, two to three days is the optimal time for imbibition of *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica.*

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