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REPRODUCTIVE BIOLOGY OF SEVEN TAXA OF *MAGNOLIA* L. IN THE SOUTH OF RUSSIAN FAR EAST

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Abstract

This paper presents the phenology of seven taxa of the genus *Magnolia* L., pollen biology on germination and storage conditions, seed productivity, germinating ability of seeds and its dependence on stratification and germination conditions. It has been found that *M. kobus* DC, *M. kobus* var. *boreales* Sarg*.*, *M. obovata* Thurb., *M. officinalis* Rehd. *et* Wils., *M. salicifolia* (Sieb. *et* Zucc.) Maxim., *M. sieboldii* K. Koch. and *M. tripetala* L. in cultivated condition produce pollen at a low viability rate (9.4 - 31.7%). Real seed productivity of the taxa being studied is less than their potential productivity. However, *M. obovata, M. officinalis* and *M. tripetala* are characterized by high seed germinating ability, up to 94%. Optimal germination conditions for seeds of *M. tripetala, M. officinalis* and *M. obovata* require protected ground (greenhouse) and stratification at 4ºC during 30 days. The high germinating ability of *M. sieboldii* seeds in the open ground is explained by stratification duration and temperature required for this species. Sarcotesta effects on higher seed germinating ability have been observed in *M. obovata* only.

Introduction

Magnolia, belonging to the family Magnoliaceae Juss. includes over 80 species, and is distributed in Southeast Asia, North and Central America (Low, 1996). The only *Magnolia* species that occurs in Russia is *Magnolia obovata* Thunb., growing in Kunashir Islands (Barkalov, 2009). Primorsky Krai has favourable climatic conditions for the cultivation of representatives of the genus *Magnolia* (Turkenya, 1991).This fact has made it possible to bring some other magnolia species under cultivation in this area.

The collection of *Magnolia* in the Botanical Garden-Institute, Far Eastern Branch of Russian Academy of Sciences (BGI FEB RAS), Vladivostok (Russia, Far East, Primorsky Krai), dates back to 1972 and at that time some seeds of *M. sieboldii* K. Koch. were brought from the Pyongyang Botanical Garden (North Korea). Forty years later, there are 20 magnolia species successfully cultivated in the Botanical Garden Institute FEB RAS.

Prospects for bringing plants under cultivation are assessed by correspondence of plant phenology with climatic conditions in which they grow. Knowledge of pollen quality, pollination and fertilization issues and production of quality seed material has both theoretical and practical importance. Pollen quality is important for prediction of seed productivity of plants and for hybridization studies (Termena, 1972; Koksheeva, 2004; Pshennikova, 2007; Liza *et al*., 2010). Production of quality seed material is an indicator of reproductive capacity of plants which depends on a number of factors: morphogenetic, genetic, physiological and ecological.

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Individual questions of reproductive biology of some species of magnolia in different climatic conditions have been studied by several authors (Minchenko and Korshuk, 1987; Kikuzawa and Mizui, 1990; Ishida, 1996; Grigorenko, 1998; Hirayama and Ishida, 2005; Korshuk and Palagecha, 2007; Setsuko *et al*., 2008; Han and Long, 2010; Wang, 2010). The present work deals with comprehensive study of the genus *Magnolia* in the Russian Far East, including the study of phenology, pollen germination, seed productivity and seed germination.

Materials and Methods

Seven taxa of *Magnolia* growing in the Botanical Garden Institute in Vladivostok (Russian Far East, Primorsky Krai) are appended in Table 1. Observations on phenology have been made using the methodology of Lapin (1967).

Table 1. List of taxa of *Magnolia* L. used in the present study.

Taxa	Locality	Origin material	h/d (m)	Beginning	
				flowering (year)	fruiting (year)
M. kobus	Central and northern Japan, southern part of the Korean Peninsula	Ukraine, Kiev, Institute of Botanical Gardens, 1984, seedlings	4.5/5.0	9	9
M. kobus var. *borealis*	Japan, Hokkaido	Ukraine, Kiev, Institute of Botanical Gardens, 1984, seedlings	05.0/5.0	14	26
M. obovata	Japan, Kuril Islands, Russia	Ukraine, Kiev, Institute of Botanical Gardens, 1986, seedlings	3.0/3.0	13	24
M. officinalis	Central China	Ukraine, Kiev, Institute of Botanical Gardens, 1989, seedlings	4.5/5.0	16	20
M. salicifolia	Central and northern Japan	Czech Republic, 1996, seeds	63.5/3.5	13	15
M. sieboldii	Japan, China, the Korean Peninsula	North Korea, Pyongyang, the Botanical Gardens, 1974, seeds	04.0/6.0	9	14
M. tripetala	The southern part of North America	Ukraine, Kiev, Institute of Botanical Gardens, 1988, seedlings	4.0/2.0	12	22

h = height of stem, d = diameter of crown of tree.

Pollen was collected during mass flowering period. Three growth medium varieties were used for study of pollen germination: 5%, 10% and 15% glucose solutions, with distilled water used for control purposes (Golubinsky, 1974). Pollen was germinated in a thermostat at 24ºC and laboratory temperature 18ºC to 20ºC. Specimens were observed through a microscope through 24h after pollen was sown. The number of germinated pollen grains was counted in five microscope fields of view for each specimen. The length of pollen tubes was measured as an indicator of pollen viability. An optimal growth medium was assumed in which germinated pollen percentage was at its highest and corresponded to maximum length of pollen tubes.

Three storage methods were used to determine optimal conditions of pollen storage: storage in laboratory conditions at 18ºC to 20ºC, storage in a household refrigerator at 4ºC and storage in a freezing cabinet at –18ºC. Pollen was stored for 7 days in tight sealed test tubes.

Seed productivity and productivity rate were determined using the method of Rabotnov (1960). Potential seed productivity (PSP) is number of seed buds per one fruit aggregate. Real seed productivity (RSP) is number of mature seeds per one fruit aggregate. Productivity rate (PR) is the ratio of potential and real seed productivity expressed in per cent. Seed productivity studies were based on 60 fruit aggregates taken for each plant. Seed and fruit parameters were also measured: length, width and weight (weight of 1,000 seeds with/without sarcotesta). Seed germinating ability was determined by sowing seeds in the open ground and in the protected ground (greenhouse) and further natural lengthy (mean air temperature in winter varying from –5ºC to –27.2ºC) stratification and artificial stratification (at 4ºC during 30 days). Also, seeds were germinated with and without sarcotesta. Studies were based on 90 seeds of each species taken for each experimental condition.

Results and Discussion

Phenology of plants:

Vegetative period of *Magnolia* species begins with swelling of generative buds (20 April 2012) at temperature around 5.4ºC. Vegetative buds swell later (5-28 May 2012). For all the seven *Magnolia* taxa studied, flowering period normally begins 10-20 days after commencement of generative bud swelling and continues for 17 to 40 days (Fig. 1A-C). The flowering of early flowering *Magnolia* species occurs before leaf unfolding period and mass flowering occurs in mid-May at 9.7ºC. Mass flowering of late flowering *Magnolia* species, which flower after leaf unfolding, occurs in mid-June at 14.5ºC. In the south of Primorsky Krai, seeds ripen in late September or in first ten-day period of October (16.5ºC). Vegetation period duration for the species being studied is 169-179 days (Table 2).

Table 2. Phenology of the genus *Magnolia* L. in cultivated condition.

Taxa	Generative bud swelling	Vegetative buds swelling	Leaf unfolding	Budding	Flowering			Fruit formation	Fruit ripening	Defoliation	Vegetation period (days)
					Start	Mass	Stop				
	Date										
M. kobus	20.04	16.05	27.05	29.04	05.05	15.05	22.05	02.10	16.10	03.11	179
M. kobus var. *borealis*	23.04	16.05	27.05	30.04	03.05	18.05	25.05	01.10	18.10	28.10	173
M. obovata	9.05	22.05	28.05	5.06	16.06	18.06	9.07	3.10	12.10	26.10	172
M. officinalis	10.05	28.05	29.05	6.06	14.06	17.06	9.07	8.10	13.10	26.10	169
M. salicifolia	21.04	05.05	10.05	28.04	03.05	19.05	03.06	02.10	13.10	25.10	175
M. sieboldii	29.04	08.05	20.05	24.05	6.06	20.06	18.07	27.09	08.10	25.10	169
M. tripetala	7.05	19.05	5.05	13.06	19.06	20.06	12.07	27.09	9.10	27.10	175

Pollen germination biology:

Seed productivity of plants is known to be largely dependent on pollen viability. Pollen quality is governed by many factors, *viz*. species particulars, climatic conditions for growing and pollen maturity. Pollen germination results for seven taxa of *Magnolia* genus on various growth mediums are shown in Table 3. Pollen germination in laboratory conditions at temperature

18-20ºC revealed that its viability does not exceed 4.8%. Because of that, further pollen germination studies were continued in a thermostat at 24ºC. This temperature increase resulted in a higher percentage of pollen viability and longer pollen tubes (Fig. 1L).

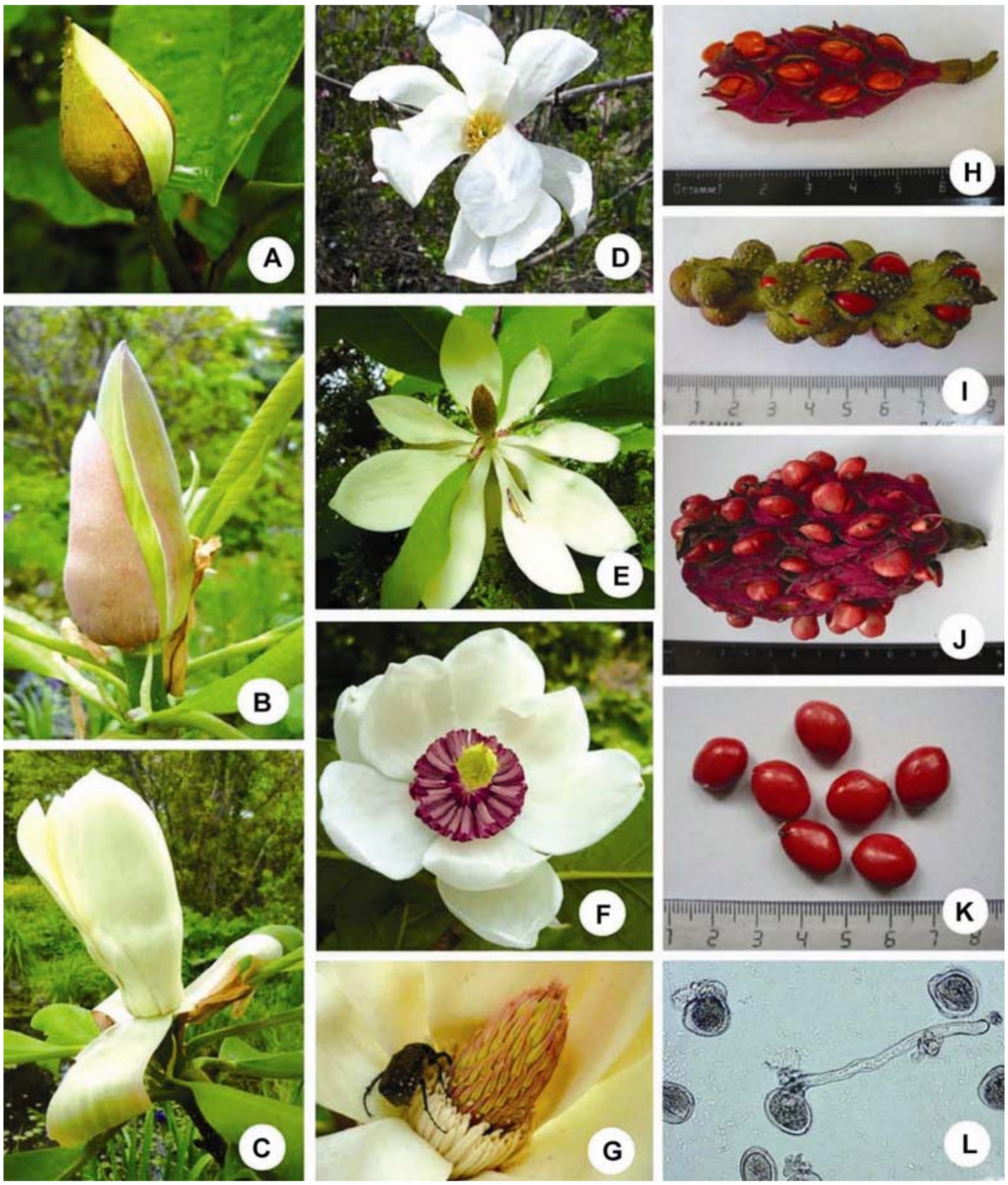

Fig. 1. Development stages of the genus *Mognolia: Magnolia sieboldii* (A. budding; F. flower; H. fruit); *Magnolia officinalis* (B,C. budding; E,G. flowers; K. seeds); *Magnolia kobus* (D. flower); *Magnolia kobus* var. *borealis* (I. fruit; L. pollen); *Magnolia obovata* (J. fruit).

Pollen germination results for *Magnolia* taxa in a thermostat at 24ºC on various growth mediums showed that the optimal medium is a 5% glucose solution in which the percentage of germinated pollen was at its highest and corresponded to the maximum length of pollen tubes. In general, the taxa being studied are characterized by low pollen viability from 9.4% to 31.7%. Among these species, highest viability data were registered for pollen of *M. kobus* var. *borealis* (31.7%) with a pollen tube length of 8.2 µm. Low pollen viability data were observed for *M. kobus* (9.4%), with its flowering period occurring in early May (9.7ºC). These data are supported by Minchenko and Korshuk (1987) showing that the main reason for poor pollen viability (in a cultivated condition in Kiev, Ukraine) may be low temperatures during flowering period preventing complete maturation of pollen.

Table 3. *Magnolia* pollen viability in different growth medium.

Taxa	Glucose concentration (%)		
	5	10	15
M. kobus	9.4±1.2 / 46±2.9	6.1±0.8 / 5.5±1.9	3.5±0.6 / 5.8±1.9
M. kobus var. *borealis*	31.7±2.8 / 34.3±2.6	10.2±2.6 / 5.8±1.8	5.2±0.6 / 4.7±1
M. obovata	11.3±1.4 / –	6.1±0.9 / –	3.4±0.6 / –
M. officinalis	13.8±1.7 / –	11.7±1.3 / –	7±0.7 / –
M. salicifolia	10.4±1.2 / 6.2±1.2	7.06±1.09 / 5.3±1.3	1.3±0.4 / 5.02±1.1
M. sieboldii	19.6±1.4 / 8.2±1.5	14.2±1.1 / 3.9±0.09	5.6±0.8 / 2.2±0.3
M. tripetalla	-	-	-

In the numerator - pollen viability (%), denominator - the length of pollen tubes (µm); «-» not examined.

Pollen ability to be stored during a long time is an important feature for hybridization studies. Results of pollen storage of *Magnolia* for different temperature conditions showed that pollen of all taxa being studied rapidly loses its viability at 18-20ºC (Fig. 2). Highest pollen viability data were observed for *M. officinalis* (8%) when stored in a freezing cabinet at –18ºC. However, this temperature of –18ºC adversely affected pollen viability during storage for the rest taxa being studied. Therefore, an optimal pollen storage condition for the majority of species is a lowered above-zero temperature of 4ºC. Pollen storage results for *Magnolia* species are confirmed by data of Minchenko and Korshuk (1987) who indicated that even five-day-long storage of magnolia pollen reduces its viability twice and more and that such pollen cannot be used for hybridization.

Seed productivity:

Results of seed productivity studies for seven taxa of *Magnolia* in a cultivated condition in the south of the Russian Far East are presented in Table 4. Potential seed productivity of the species being studied is defined by the number of seed buds per one fruit aggregate, being an upper limit of a species' seed productivity, and characterizes its potential that is little dependent on environmental conditions. Potential seed productivity varies depending on species and is equal to 48-210 ovules per one fruit aggregate. Real seed productivity was found by the number of seeds beginning to develop in a fruit. It amounted up to 55 seeds per one fruit aggregate which is much lower than potential seed productivity. Despite low real seed productivity, productivity rate for *M. sieboldii* and *M. tripetala* is above 50%. The lowest productivity rate was registered for *M. salicifolia* (1.7%).

Table 4. Seed productivity of seven taxa of *Magnolia* in cultivated condition.

Taxa	PSP			RSP			PR (%)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
M. kobus	26	66	74	9	31	11	15.4	61.3	14,8
M. kobus var. *borealis*	50	78	62	2	32	15	10	55.1	24,2
M. obovata	36	158	120	9	69	47	11.2	47.8	39.1
M. officinalis	166	240	210	6	65	31	8.4	25.3	14.7
M. salicifolia	40	55	60	-	1	-	-	1.7	-
M. sieboldii	26	52	48	6	39	28	13.4	64.2	58.2
M. tripetala	74	112	96	4	86	55	4.1	86.8	57.5

PSP = potential seed productivity, RSP = real seed productivity, PR = productivity rate.

Characteristics of seeds and fruits of *Magnolia* taxa are presented in Table 5. Large fruit aggregates (8.7-15.0 cm) and seeds (0.8-1.3 cm) are typical in *M. officinalis, M. obovata* and *M. tripetala*, while small ones (3.2 cm) are observed in *M. salicifolia* (Fig. 1H-K).

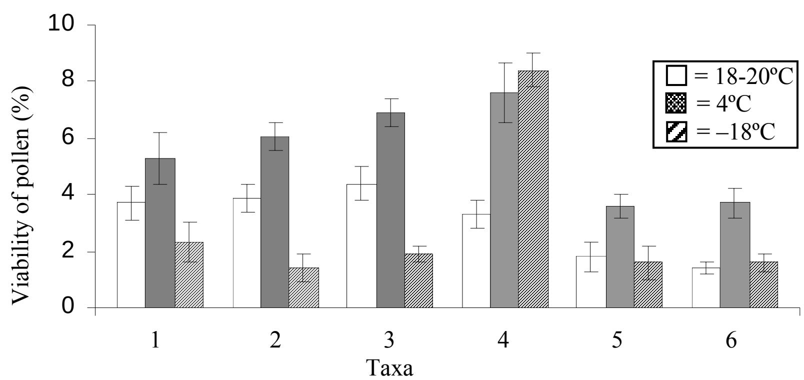

Fig. 2. Viability of *Magnolia* pollen after storage at different temperatures. 1. *M. kobus*, 2. *M. kobus* var. *borealis*, 3. M. *obovata*, 4. *M. officinalis,* 5. *M. salicifolia*, 6. *M. sieboldii*.

Seed germination:

Seed germination of *Magnolia* taxa in a cultivated condition in the south of Russian Far East showed that high germinating ability (67-94%) is typical in *M. tripetala, M. officinalis* and *M. obovata* (Table 6). Despite a high productivity rate of *M. kobus*, germinating ability of its seeds is low, 3.0% to 4.4%. Seed germination in the open ground and in the protected ground showed that optimal conditions for seeds of *M. tripetala, M. officinalis* and *M. obovata* are artificial stratification at 4ºC during 30 days and further germination in the protected ground. Optimal conditions for *M. sieboldii* seeds are in the open ground (long-time stratification at 0ºC to –27ºC).

The present study revealed that a favourable effect on seed germinating ability was observed for *M. obovata* only 57.3% without sarcotesta and 94.3% with sarcotesta. No sarcotesta effects on seed germination were registered for other species.

Table 5. Morphometric characteristics of the fruits and seeds of seven taxa of *Magnolia*.

Taxa	Fruit			Seed			
	Length (cm)	Width (cm)	Weight (g)	Length (cm)	Width (cm)	Weight (S) 1000 (g)	Weight (WS) 1000 (g)
M. kobus	7.2 ± 0.8	2.1 ± 0.06	9.5 ± 0.7	0.9 ± 0.01	0.8 ± 0.02	323	169
M. kobus var. *borealis*	4.5 ± 0.1	2 ± 0.1	8.9 ± 0.9	0.8 ± 0.02	0.7 ± 0.02	226.9	122
M. obovata	8.7 ± 0.3	4.6 ± 0.12	43.3 ± 3.3	1.1 ± 0.02	0.9 ± 0.01	262.5	152.6
M. officinalis	15 ± 0.5	4.9 ± 0.07	108.8 ± 8.5	1.3 ± 0.01	1.1 ± 0.02	528.2	399.3
M. salicifolia	3.2 ± 0.7	1.2 ± 0.6	2.2 ± 0.4	0.8 ± 0.01	0.7 ± 0.01	–	–
M. sieboldii	5.3 ± 0.9	2.07 ± 0.02	3.4 ± 0.1	0.5 ± 0.02	0.48 ± 0	52.7	39.5
M. tripetala	7.2 ± 0.2	3.9 ± 0.09	40.3 ± 2.2	0.8 ± 0.02	0.7 ± 0	151.98	99.5

S = seeds with sarkotesta, WS = seeds without sarkotesta, – = not examined.

Table 6. Seed germination of seven taxa of *Magnolia* in different condition.

Taxa	% of seeds germination in greenhouse		% of seed germination in the open ground	
	with sarcotesta	without sarcotesta	with sarcotesta	without sarcotesta
M. kobus	3	0	4.4 ± 0.1	0
M. kobus var. *borealis*	0	0	0	0
M. obovata	94.3 ± 5.7	57.3 ± 4.7	59.8 ± 12.6	45.5 ± 7.8
M. officinalis	70.1 ± 11,8	47.3 ± 1,3	61±5.9	45.5 ± 6.1
M. salicifolia	–	–	–	–
M. sieboldii	37.7 ± 2.3	56.7 ± 5.7	42.4 ± 3.9	52 ± 6.1
M. tripetala	67.3 ± 11.6	56 ± 5.8	66.6 ± 15.04	47.8 ± 9.4

‘0’ denotes not germinated, ‘–’ denotes not examined.

The present study addresses phenology of seven taxa of the genus *Magnolia* L., pollen biology of germination and storage conditions, seed productivity, germinating ability of seeds and its dependence on stratification and germination conditions. It was found that the flowering period of the taxa being studied occurs in May – June at a time of low air temperatures varying from 9ºC to 14.5ºC, which has adverse effects on pollen viability. Due to this circumstance, *M. kobus*, *M. obovata, M. officinalis, M. tripetala, M. salicifolia* and *M. sieboldii* are characterized by low pollen fertility (9.4-31.7%) in the south of the Russian Far East which affects their seed productivity. Real seed productivity of these taxa is considerably lower (1-55 seeds per one fruit aggregate) than their potential seed productivity (48-210 ovules per one fruit aggregate). Despite their low pollen viability and productivity rate, *M. tripetala, M. officinalis* and *M. obovata* in a cultivated condition produce seeds with high germinating ability (67-94%). At the same time, *M. sieboldii* features a high productivity rate but low seed germinating ability. Seed germination results showed that optimal conditions for species with high germinating ability such as *M. tripetala, M. officinalis* and *M. obovata* are artificial stratification, presence of sarcotesta and protected ground conditions. We believe that low germinating ability of *M. sieboldii* and *M. kobus* in the protected ground can be explained by stratification duration and temperature. Sarcotesta effects on higher seed geminating ability were observed for *M. obovata* only.

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