Korean J. Plant Res. 27(3):256-262(2014)
http://dx.doi.org/10.7732/kjpr.2014.27.3.256

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Effect of Harvesting Time and Storage Duration of *Viscum album* Seeds on *in vitro* and *ex vitro* Germination on the Branch of *Prunus mume*

Hyun Woo Lee[1], Amal Kumar Ghimeray[1], Bo-Duk Lee[1], Pankaja Sharma[1], Ie Sung Shim[2] and Cheol Ho Park[1]*

[1]Department of Bio-health Technology, Kangwon National University, Chuncheon 200-701, Korea
[2]Departments of Horticulture, Seoul City University, Seoul 130-743, Korea

Abstract - *Viscum album* var. *coloratum* (mistletoe) is considered as one of the endangered plant species in Korea. Our objective is to restore its population and multiplication of plant by *ex situ* method. In this research we explored the maximum germination (*in vitro*) of freshly harvested and stored seeds of mistletoe collected in different time intervals. *Ex vitro* germination after artificial inoculation on the branches of *Prunus mume* in different physiological conditions was also monitored. The research revealed that the lately harvested seeds (Feb. and March 2014) were superior over early harvested seeds (Nov. 2013 and Jan. 2014) of mistletoe due to the higher percentage of germination (above 93%). According to the data, it is also revealed that the survival and germination rate of mistletoe seeds decreased with the increase in storage duration. In *ex vitro* germination, the fluctuated temperature of a glass house in natural condition enhanced (four fold) the rate of germination on the branches of *Prunus mume* than the constant temperature condition in the glass house.

Key words - *Ex vitro* germination, Seed harvesting, Mistletoe, *Prunus mume*, *Viscum album*

Introduction

Several species of plants are getting endangered or threatened due to over-exploitation, habitat destruction, urbanization, disease, pollution, introduction of exotic species, climate change, etc. To preserve the rare species, *ex situ* conservation technique is one of the best accepted methods, which involves the preservation and maintenance of endangered species outside their natural habitat, in the form of seed, whole plants, somatic tissues, gametes, pollen etc. (IUCN, 2002).

Mistletoes are angisopermic, hemiparasitic plants in several families in the order Santalales. It has been reported that mistletoe has more than 1400 species in the four families of Loranthaceae, Misodendradraceae, Santalaceae and Viscaceae; among which Loranthaceae is the largest family with 900 or more species (Reid *et al.,* 1995, Nickrent 2002, Kim *et al.,* 2013). In Korea there are four species and four genera in the two families; Santalaceae and Loranthaceae (Kim 2007a; Kim 2007b) and distributed all over the peninsula (Huaxing *et al.,* 2003). In the Loranthaceae family, *Viscum album* var. *coloratum* (Kom. Ohwi) is considered as one of the endangered species in Korea that grows on the branches or twigs of different host plants like *Quercus and Morus* species (Kim *et al.,* 2013; Lee *et al.,* 2010b). The plant develops a special structure called haustorium, which penetrates into the phloem tissue of the host plant to uptake water and nutrients (Kim *et al*., 2013, Calvin and Wilson 2006; Lee *et al.,* 2009).

In recent years, populations of endangered mistletoe (*Viscum album* var. *coloratum* (Kom. Ohwi) have been declining due to over exploitation, habitat destruction and climate change. Therefore, our objective is to restore its population and multiplication of the plant by *ex situ* method. Previously, Lee *et al.,* (2010b) researched on the suitability of mistletoe host and found that *Malus pumila* var. *dulcissima* and *Quercus mongolica* trees were good host for the mistletoe growth *ex vitro*. In this study, our objective is (a) to study the maximum germination and survival rate of mistletoe harvested in different time intervals, (b) to monitor the effect of storage duration on germination and survivability *in vitro* and (c) to examine *ex vitro* germination after artificial inoculation on the branches of *Prunus mume* in different physiological conditions.

*Corresponding author. E-mail : chpark@kangwon.ac.kr

Materials and methods

Sample collection

Mistletoe (*Viscum album* var. *Coloratum* (Kom. Ohwi) yellow fruits grown in the oak trees (*Quercus mongolica)* in natural habitat were harvested on November 2013, January 2014, February 2014 and March 2014 from Yuljeon-RI, Hongcheon-gun, Gangwon-do (37.74N, 128.32E), South Korea.

Storage conditions of mistletoe seeds

The mistletoe fruits harvested freshly at different times are considered as non-after-ripened seeds were subjected to the germination test (30 seeds in 3 replications). Remaining fruits were stored at 0°C for 1, 2 and 3 months and considered as after-ripened seed.

Sterilization, *in vitro* germination and survivability test

The pulp of the freshly harvested fruits was removed manually by rubbing them against a paper towel. To avoid contamination from microorganisms the washing treatment was performed on 30 seeds by immersing in 50 ml of sodium hypochlorite solution (1.5%) for 3 min and was shaken vigorously. The seeds were filtered out of solution and washed with six changes of deionized water. Another 30 seeds were washed only with deionized water for six changes which served as control. After draining out the liquid of the final wash, three replicates of 30 seeds were placed on absorbent paper that had been moistened with deionized water in petri dishes (9-cm). The petri dishes were then placed at two constant temperature (15°C and 22 ± 2°C) regimes in the germinator. During the germination period, petri dishes were watered as needed with distilled water to ensure adequate moisture for seed germination. At periodic intervals of a week, the seeds in the dishes were observed for germination and after 5 weeks, the germination percentage of each treatment was calculated from the average of 3 replications' percentage**.** Likewise, the survivability (survival rates) were evaluated every week based by counting the remaining individuals whose seed coat color was not changed from green to brown. The after-ripened seeds (stored for 3, 2, and 1 month) were also followed the same procedures for the germination test as of non-after ripened seeds (Fig. 1 A, B and C).

Artificial inoculation and *ex vitro* germination

Mistletoe seeds were inoculated artificially on the branches of *Prunus mume* in a glass house where the temperature was maintained (24 ± 2°C, average humidity 39.6%). For comparison, mistletoe seeds were also inoculated on the branches of *Prunus mume* in a glass house where the temperature was not maintained (fluctuating temperature of maximum 22.3 and minimum -1.2°C (average temperature 11.8°C, average humidity 32.5%). Further, to understand the physiological effect of *ex vitro* germination of seeds, an experiment was designed where some of the branches of *Prunus mume* were artificially inoculated with mistletoe seeds and covered partially and fully by polyethylene bottles in order to control humidity on branches. The uncovered (natural condition) branches were served as a control. The maximum, minimum temperature and humidity were checked regularly and *ex vitro* germination and survivability of seeds was investigated for 8 weeks at the interval of 1 week.

Statistical analysis

The data on germination (%) and survival rate (%) were subjected to ANOVA using IBM SPSS Advanced Statistics 20. The treatment means were tested by Tukey tests at the 5% level of significance.

Results and Discussion

Survivability and Germination performance by freshly harvested seeds *in vitro*

The freshly harvested *Viscum album* (mistletoe) seeds when allowed to germinate in petri dish showed highest survival rate (Table 1). The survivability tests were performed at two constant temperature regimes of 15 and 22 ± 2°C and the survival rate were in the range of 93 to 100% in both temperature regimes. There were no significant differences in survivability between the seeds harvested in different months of the year. Our result can be comparable with the previous findings by Scharf and Robort (1970), where they reported the high viability of freshly harvested seeds of two species of dwarf

Table 1. Survival rate of freshly harvested mistletoe seeds at different time intervals (Nov. 2013, Jan. 2014, Feb. 2014 and March 2014) as determined at 15 and 22 ± 2°C

Average number of seeds survived								
Sample (harvested time)	Temperature (°C)	No. of seeds used in 3 replications	7 days	14 days	21 days	28 days	35 days	Total Survival rate (%) after 35 days
Nov. 2013	15	30	30	30	30	30	30	100[a]
	22	30	30	30	29	29	29	96.7[a]
Jan. 2014	15	30	30	30	30	30	30	100[a]
	22	30	30	30	29	29	28	93.3[a]
Feb. 2014	15	30	30	30	30	30	30	100[a]
	22	30	30	29	29	29	29	96.7[a]
March 2014	15	30	30	30	30	30	30	100[a]
	22	30	30	30	30	29	29	96.7[a]

Values are given as mean of 3 replicates. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

Table 2. Germination rate of freshly harvested mistletoe seeds at different time intervals (Nov. 2013, Jan. 2014 and Feb. 2014 and March 2014) as determined at 15 and 22 ± 2°C

Number of Germinations									
Sample (harvested time)	Temp. (°C)	No. of seeds used in 3 replications	7 days	14 days	21 days	28 days	35 days	Total No. of Germination	Total germination rate (%) after 35 days
Nov. 2013	15	30	0	1	6	2	1	10	33.3[c]
	22	30	0	2	7	3	1	13	43.3[c]
Jan. 2014	15	30	0	1	9	6	2	18	60[b]
	22	30	0	2	8	6	1	17	56.7b[c]
Feb. 2014	15	30	0	3	10	10	3	26	86.7[a]
	22	30	0	4	10	8	3	25	83.3[ab]
March 2014	15	30	0	2	10	11	4	27	86.7[a]
	22	30	0	3	14	7	2	26	86.7[a]

Values are given as mean of 3 replicates. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

mistletoe *Arceuthobium abietinum* and *A. occidentale.* They also reported that the viability not significantly influenced by the year of collection, place of collection or host plant from which collected.

The seeds harvested in different period of the year showed a significantly different germination pattern when observed at two constant temperature regimes of 15 and 22 ± 2°C (Table 2, Fig. 1 D). The seeds freshly harvested on February and March 2014 showed higher rate of germination (83.3 to 86.7%). However, the early harvested seeds in the month of Nov. 2013 and Jan. 2014 showed lower germination percentage which were in the range of 33.3 to 43.3 and 56.7 to 60% respectively. This lower percentage of germination showed by early harvested seeds could be due to the lack of full maturation of some seeds during early harvesting time, or, there could be other environmental conditions (like temperature etc.) that affect the germination. According to Scharf and Robort (1970), temperature significantly affected the rate and percentage of mistletoe seed germination.

Effect of storage temperature on germination and survival rate of mistletoe seeds *in vitro*

The storage duration significantly affects the survival rate of mistletoe seeds *in vitro* (Table 3). The survival rate of seeds was decreased with the increase in storage duration. The seeds stored for a month showed 93.3 and 83.3% of

Table 3. Survival rate of stored mistletoe seeds for 3 months, 2 months and 1 month as determined by germination test at 15 and 22 ± 2°C

Average number of seed survived								
Stored duration	Temperature (°C)	No. of seeds used in 3 replications	7 days	14 days	21 days	28 days	35 days	Average survival rate (%) after 35 days
1 month	15	30	30	30	30	29	28	93.3^{a}
	22	30	29	27	27	25	25	83.3^{ab}
2 months	15	30	29	27	26	24	23	76.6^{b}
	22	30	25	21	13	13	12	40^{c}
3 months	15	30	19	8	3	1	0	0^{d}
	22	30	25	12	3	1	0	0^{d}

Values are given as mean of 3 replicates. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

Table 4. Germination rate of stored mistletoe seeds for 3 months, 2 months and 1 month as determined by germination test at 15 and 22 ± 2°C

Number of Germinations									
Sample	Temperature (°C)	No. of seeds used in 3 replications	7 days	14 days	21 days	28 days	35 days	Total No. of Germination	Germination rate (%)
1 month	15	30	0	2	8	10	4	24	80^{a}
	22	30	0	5	10	9	1	25	83.3^{a}
2 months	15	30	0	0	5	6	2	13	43.3^{b}
	22	30	0	4	7	0	0	11	36.6^{b}
3 months	15	30	0	0	0	0	0	0	0^{c}
	22	30	0	0	0	0	0	0	0^{c}

Values are given as mean of 3 replicates. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

survivability as observed in 15 and 22 ± 2°C germinator respectively. However, the survivability decreased to 76.6% when the seed was stored for 2 months. Furthermore, the survivability was decreased to 0% when the seeds were stored for 3 months. The germination rate was also affected significantly by the storage conditions (Table 4). One month stored seeds showed 80 to 83.3% germination; however, the percentage decreased to 36.6 in seeds stored for two months. Further, in 3 months stored seed the germination rate was reduced to 0%. In our research, the stored (at 0°C) seeds gradually lost their viability and germination rate that could be due to 'injury and destruction' of the embryo (Heinricher 1915), and somehow, also due to deterioration by mold fungi (Wicker 1967).

Germination and Survival rate of mistletoe seeds after inoculation

The survival rate of mistletoe seed in uncovered (natural condition), partially covered and fully covered *Prunus mume* branches by polyethylene bottles (Fig. 2 A and B) were 40, 20 and 0% respectively in a glass house where the average constant temperature and humidity was 24 ± 2°C and 39.6% respectively (Table 5). On the other hand, the survival rate was 60, 63.3 and 0% for uncovered (natural condition), partially covered and fully covered by polyethylene bottles respectively in a glass house (temperature not maintained) where the maximum, minimum temperature and humidity was 22.3°C, -1.2°C and 32.5% respectively. According to the data (Table 6), the germination percentage of mistletoe seeds were higher in a temperature not maintained glass house with 60% in natural conditions (uncovered by polyethylene), and 56.7% germination was observed in partially covered branches, but in fully covered branches the germination rate was reduced to 0%. Similarly, the germination rate of mistletoe seed in uncovered (natural condition), partially covered and fully covered *Prunus mume* branches by polyethylene bottles were 16.7, 13.3 and 0%, respectively inside a glass house

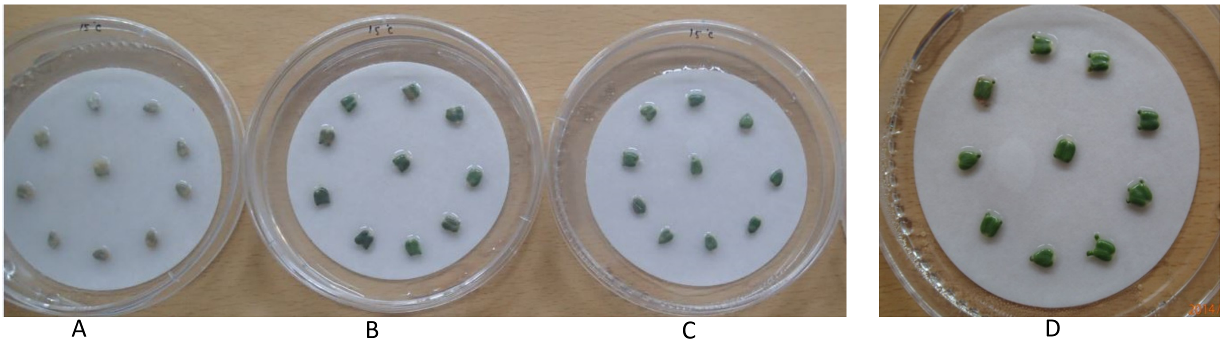

Fig. 1. A, B and C represent *in vitro* germination of mistletoe seeds stored for 3, 2, and 1 months respectively. ‘D’ represents higher percentage of germination shown by mistletoe seeds freshly harvested on the month of early march 2014.

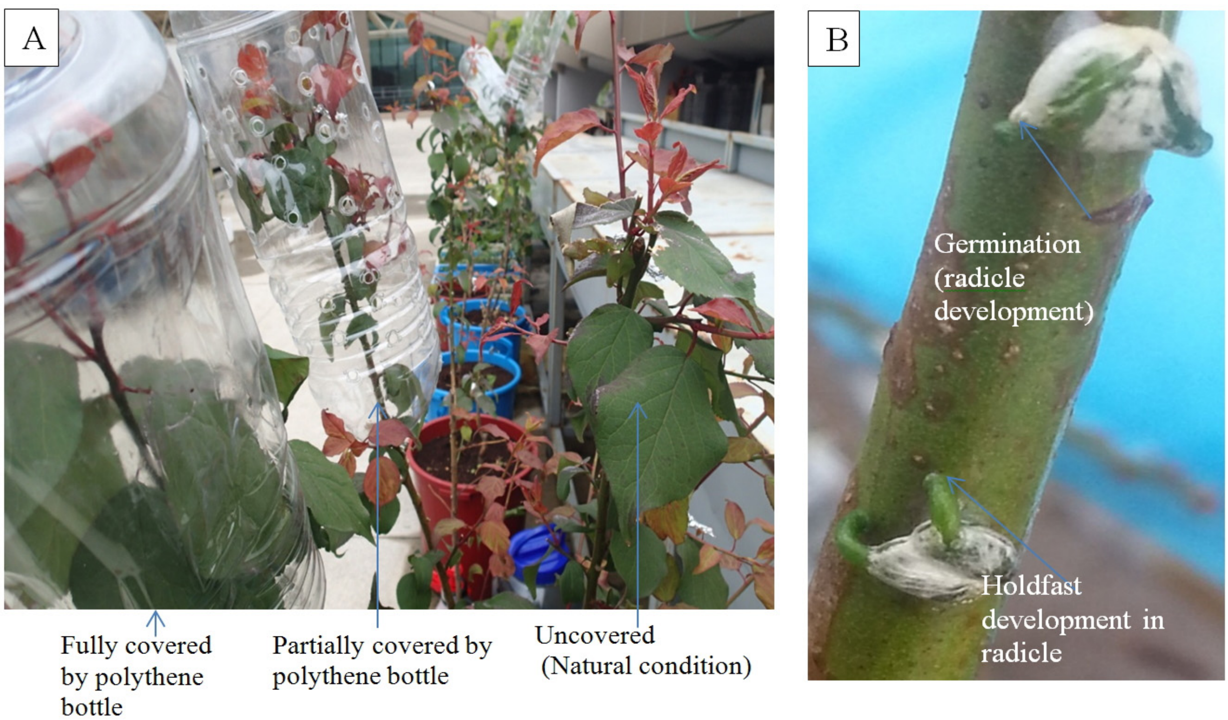

Fig. 2. (A) *Ex vitro* germination performance of mistletoe seeds after artificial inoculation on the branches of *Prunus mume* inside glass house in different physiological conditions. (B) Close view of Mistletoe seed germination and radicle development on the branch of *Prunus mume*.

where temperature was fully maintained.

In conclusion, the lately harvested seeds on Feb. and March 2014 were superior over early harvested seeds on Nov. 2013 and Jan. 2014 of mistletoe because of higher percentage of germination. From this research, it is also observed that the survival and germination rate of mistletoe seeds decreased with the increase in storage duration. In a previous research, *Scharpf and Parmeter* (1962) also found that the stored (in freezing temperature) seeds of *A. occidentale* (kind of mistletoe) decreased the germination percentage. In *ex vitro* germination, temperature and humidity played an important role to induce survivability and germination rate of mistletoe seeds. From this research it is also confirmed that the fluctuated temperature in natural condition (maximum, minimum temperature and humidity was 22.3°C, -1.2°C and 32.5% respectively) in a glass house enhanced the rate of germination on the branches of *Prunus mume* than the constant temperature condition (24 ± 2°C) in the glass house.

Table 5. Survival rate of mistletoe seeds after artificial inoculation on the branch of *Prunus mume*

Conditions		Temp. (°C)	Humidity (%)	No. of seeds used	1w	2w	3w	4w	5w	6w	7w	8w	Mean survival rate (%) after 8W
				Average number of seed survived									
maintained glass house	Uncovered (natural condition)	23.6	39.6	30	20	13	13	12	12	12	12	12	40^{b}
	Partially covered	24.9	40.7	30	30	19	9	8	6	6	6	6	20^{c}
	Fully covered	25.4	81.3	30	3	0	0	0	0	0	0	0	0^{d}
not maintained-glass house	Uncovered (natural ondition)	11.8	32.5	30	29	27	22	22	20	19	19	18	60.0^{a}
	Partially covered	11.4	34.7	30	29	28	26	23	19	19	19	19	63.3^{a}
	Fully covered	10.9	70.4	30	24	14	1	0	0	0	0	0	0^{d}

The test was carried out in a temperature maintained and not maintained glass house.
Values are given as mean of 3 replicates. 'w' in the table represent week. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

Table 6. Germination rate of mistletoe seeds after artificial inoculation on the branch of *Prunus mume*

Conditions		Temp. (°C)	Humidity (%)	No. of seeds used	1w	2w	3w	4w	5w	6w	7w	8w	Total No. of Germ.	Mean rate (%) after 8W
				Number of Germinations										
maintained glass house	uncovered (natural condition)	22.6	39.6	30	0	0	1	0	2	0	1	1	5	16.7^{b}
	Partially covered	23.9	40.7	30	0	0	0	1	0	1	2	0	4	13.3^{b}
	Fully covered	24.4	81.3	30	0	0	0	0	0	0	0	0	0	0^{c}
not maintained-glass house	uncovered (natural condition)	11.8	32.5	30	0	0	0	1	2	3	8	3	18	60^{a}
	Partially covered	11.4	34.7	30	0	0	1	1	3	4	6	2	17	56.7^{a}
	Fully covered	10.9	70.4	30	0	0	0	0	0	0	0	0	0	0^{c}

The test was carried out in a temperature maintained and not maintained glass house.
Values are given as mean of 3 replicates. 'w' in the table represent week. Small letters in superscripts represent significant differences at the level of 5% according to Tukey Test.

Acknowledgement

Authors appreciate Korea Forest Service (project number C1010036-01-01) and the Institute of Bioscience and Biotechnology, College of Biomedical Sciences, Kangwon National University for supporting research funds and providing facilities.

References

Calvin, C.L. and C.A. Wilson. 2006. Comparative morphology of epicortical roots in old and new world Loranthaceae with reference to root types, origin, patterns of longitudinal extension and potential for clonal growth. Flora 201:345-353.

Huaxing, Q., C.H. Hsing, K.H. Xing and M.G. Gilbert. 2003. Loranthaceae. Flora of China 5: 220-239.

Heinricher, E. 1915. Ueber besondere Keimungsbedingungen welche die Samen der Zwergmistel, *Arceuthobium oxycedri* (D.C.) M. Bieb., beanspruchen. Centralbl. Bakt. 42:705-711.

IUCN technical guidelines on the management of *ex situ* populations for conservation. 14th meeting of the programme committee of council, Gland Switzerland, 10th Dec. 2002.

Kim, Y.D. 2007a. Santalaceae R.Br. The Genera of Vascular Plants of Korea. Park, C.-W. (ed.), Academy Publishing Co., Seoul, Korea. pp. 653-655.

Kim, Y.D. 2007b. Loranthaceae Juss. The Genera of Vascular Plants of Korea. Park, C.W. (ed.), Academy Publishing Co., Seoul, Korea. pp. 656-657.

Kim, C.S., S.Y. Kim., B.Y. Sun and J.S. Yi. 2013. A review of the taxonomic and ecological characteristics of Korean mistletoe types (*Viscum, Korthalsella, Loranthus* and *Taxillus*). Korean J. Plant Taxon. 43:81-89.

Lee, S.G., S.H. Lee and H. Kang. 2010a. Effect of storage germination duration, medium and viscin on *in vitro* seed germination in endangered species, *Loranthus tanakae*. Korean J. For. Soc. 99:618-624.

Lee, S.G., S.H. Lee, K.W. Park, Y.H. Kwon and H. Kang. 2010b. Effects of host tree species, temperature and humidity on *ex-vitro* seed germination in endangered species of *Loranthus tanakae*. Korean J. For. Soc. 99:871-877.

Lee, B.D. and B.S. Park. 2009. Anatomical characteristics of Korean mistletoe (*Viscum album* var. *coloratum* (Kom.) Ohwi) stem. Korean J. Plant Res. 22:287-292.

Nickrent, D. L. 2002. Santalales (Misletoe). Encyclopedia of Life Science, John Wiley & Sons, New York, USA. pp. 1-4.

Reid, N., M.S. Smith and Z. Yan. 1995. Ecology and population biology of mistletoes, in Forest Canopies (Lawman, M.D. and Nadkarni, N.M., eds). Academic Press. Massachusetts, USA. pp. 285-310.

Scharpf, R.F. and J.R. Parmeter. 1962. The collection, storage and germination of seeds of dwarf mistletoe. J. Forestry. 60:551-552.

Wicker, Ed. F. 1962. Seed collection and storage for *Arceuthobium spp*. U.S. Forest Service., Intermountain Forest & Range Exp. Sta., Research paper INT-33. p. 13.

(Received 14 April 2014 ; Revised 19 June 2014 ; Accepted 23 June 2014)