Korean J. Plant Res. 28(6):710-717(2015)
http://dx.doi.org/10.7732/kjpr.2015.28.6.710

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

The Impact of Environmental and Host Specificity in Seed Germination and Survival of Korean Mistletoe [*Viscum album* var. *coloratum* (Kom.) Ohwi]

Bo Duck Lee[1], Young Woo Lee[2], Seong Min Kim[3], Hyo Cheng Cheng[3] and Ie Sung Shim[3]*

[1]Department of Horticulture, Kangwon National University, Chuncheon 24341, Korea
[2]Graduate School, Purdue University, West Lafayette, IN 47907, USA
[3]Department of Environmental Horticulture, University of Seoul, Seoul 02504, Korea

Abstract - Humankind has been searching for medicinal materials from various plant sources in an attempt to treat disease. Mistletoe is one indubitable plant source for these materials due to its effectiveness in treating various diseases, but it has almost disappeared from the mountainous areas of Korea due to excessive harvesting. In this study, in order to select host tree species for Korean mistletoe [*Viscum album* var. *coloratum* (Kom.) Ohwi] by seed inoculation and to clarify the effect of host specificity among various tree species were conducted for the purpose of gaining basic information for the artificial cultivation of Korean mistletoe. Almost all the seeds of Korean mistletoe germinated *in vitro* at the temperature of 15℃. Among host trees used in this study, *Prunus mume* showed the highest parasitic affinity with inoculated Korean mistletoe, compared with any other host plants. However, treatment of hormones could not increase the low survival rate of Korean mistletoe on the host trees.

Key words - Artificial propagation, Haustorium, Plant hormone, Inoculation, *Prunus mume*

Introduction

As an evergreen parasitic plant, *Viscum album* forms adventitious roots to absorb water and nutrients from its host plant for photosynthesis (Visser, 1981). In Korea, *V. album* has a hemiparasitic relationship with *Quercus spp*. (e.g. *Quercus dentata*), *Celtis sinensis*, *Betula platyphylla* var. *japonica*, *Salix koreensis*, *Alnus japonica*, and *Castanea crenata* var. *dulcis* (Kim, 1996; Lee, 1985; Ministry of Agriculture, 2000; Ministry of Agriculture, 2007). Interestingly, Lee (2009) reported that Korean mistletoe [*Viscum album* var. *coloratum* (Kom.) Ohwi] was observed on both autogenously grown conifers including *Pinus densiflora* and *Pinus koraiensis*, and on naturally grown on broad-leaved trees, such as *Zelkova serrata*, *Diospyros kaki*, *Acer mono*, *Acer palmatum*, *Morus alba*, *Kalopanax pictus*, *Ginkgo biloba,* in the regions where a colony of mistletoe was observed. It is widely known that host specificity of Korean mistletoe have a lot of species of trees. According to Hariri *et al*. (1991), however, host plant may have certain defense mechanisms that will be generating during invasion of endophyte. In forming a physical connection between the mistletoe and host organization, it is suspected that presence of the chemical defense mechanism. This result suggests that Korean mistletoe may have host specificity.

V. album has long been used as a medicine for various diseases; lectins and viscotoxins found in the plant are well-known as biologically active proteins and polypeptides (Machaidze *et al*., 1996; Tubeuf, 1923). Recently, there has been increasing attention to its efficacy in chemotherapy; mistletoe extracts are being used as a new anti-cancer substance. Anti-cancer activity of the mistletoe has been reported due to the cytotoxic and immunomodulating substances (Büssing, 1999, 2000). In Korea, many studies for metabolite identification and pharmacological actions of mistletoe extracts have been conducted (Kim, 1997; Park, 1999; Sung, 2005; Yoon, 1997; Lee, 2002; Lee, 2003; Jang, 1997; Jun, 2004; Jung, 1999; Jung, 2005; Choi, 2005). By comparing Korean and European mistletoe, it was discovered that the two species

*Corresponding author. E-mail : isshim@uos.ac.kr
Tel. +82-2-6490-2688

have different lectin components, which cause different antibody responses (Lyu *et al*., 2000). Choi (2005) reported that DNA sequence alignments between the Korean and European mistletoe did not correspond to each other. Lee (2003) also demonstrated, through research measuring physiological activities from four different varieties, that metabolite compositions in mistletoe extract depend on the host plant.

Plant growth regulators are adjustable to a particular growth response in plant; mainly it represents the plant hormone. The plant hormone auxin and cytokinine promote the plant growth and differentiation of cell. It has been known that control of development of shooting and rooting is depending on the rate of auxin and cytokinine. According to the experiment of Martin *et al*. (2012) that treated various concentrations of IAA and kinetin to mistletoe, health of mistletoe explant was related to the concentration of IAA. At an IAA/kinetin ratio of 0.1, also, mistletoe explant was healthiest.

As mistletoe efficacy has been demonstrated in the medical field, and the need for dietary supplements has increased, mistletoe has been indiscriminately taken by people picking medical herbs so that autogenously grown mistletoe cannot be found easily, even in some national parks. Therefore, there is a strong demand for artificial cultivation of mistletoe due to the depletion of natural mistletoe. In order to accumulate fundamental information for the development of artificial mistletoe propagation, we conducted inoculation experiments of mistletoe seeds to investigate host affinity and to verify the parasitic mechanism.

Materials and Methods

Seed collection and germination of Korean mistletoe

To collect Korean mistletoe seeds, naturally grown 25-years-old *Viscum album* var. *coloratum* (Kom.) Ohwi seeds were taken from an approximately 35-years-old *Quercus variabilis* host tree growing on Mt. Jiri (Sannae-myeon, Namwon). To check the result of Lamont (1983) that 15℃ was the optimal temperature for mistletoe seed germination, comparative germination tests were carried out with the ambient temperature. 25 mistletoe seeds were placed on filter paper wetted with distilled water in each of the two petri dishes with a cover and incubated to keep relative humidity at 70% in chamber setting 15 and 25℃. This test designated as one replication and the test was performed five times. After three weeks, seeds with hypocotyls that were 1 ㎜ or longer were counted as germinated seeds.

Host specificity of Korean mistletoe seed

Based on the previous report on mistletoe habitats conducted by Lee (2009), *Quercus variabilis*, *Castanea crenata* var. *dulcis*, *Prunus serrulata* var. *spontanea*, *Prunus mume*, and *Malus pumila* var. *dulcissima* were selected as potential candidates to determine host affinity with artificially inoculated mistletoe seeds. Mistletoe seeds were taken from an approximately 20-year-old *Viscum album* var. *coloratum* (Kom.) Ohwi parasitizing on a *Quercus variabilis* tree on Mt. Jiri (Sannaemyeon, Namwon) on February 28, 2005. Before this test, seeds were stored at -2℃ for 15 days. For seed inoculation, the mistletoe pericarp was manually removed and 10 peeled seeds were then inoculated at 10- ㎝ intervals, via the adhesiveness of their internal flesh, onto the two-year-old branches of the five host plants. The inoculated branches of each host were designated as one replication and the test was performed thrice. This inoculation was conducted in the experimental field installed in Susanri area (Sudong-myeon, Namyangju). In the spring of the next year after inoculation, the degrees of affinity were calculated by investigating whether cotyledon grew from the parasitic seeds.

Field test for mistletoe seed inoculation

From the five species in the host specificity test, *Prunus mume* was selected as a host tree for the field test of seed inoculation. Also, *Morus alba* was selected as a control their medium-sized heights are suitable for relatively easy management. Two-meter-tall *P. mume* and *M. alba,* grown for three years after their propagation, were transplanted in experimental city (Sudong-myeon, Namyangju) for this seed inoculation test. Seeds were collected from an approximately 20-year-old *Viscum album* var. *coloratum* (Kom.) Ohwi parasitizing on a *Quercus variabilis* tree on Mt. Jiri (Sannaemyeon, Namwon) at the end of February 2006, and stored at -2℃ for 15 days. The mistletoe pericarp was manually removed and 5 peeled seeds were then inoculated at 10- ㎝ intervals, via the adhesiveness of their internal flesh, onto the two-year-old branches of the host plants. The inoculated branches of each host were designated as one replication and the test was performed five times. After the first year following inoculation, seed germination on the bark of host plants and hypocotyl elongation were investigated. The survival rates of mistletoe seeds were investigated during the spring of the second year. At the following fall, survival rates of germinated seeds were finally judged. Three years after inoculation, the numbers of mistletoe branches were counted.

Effects of hormone treatment for survival

To investigate the effect of hormones on the survival of hypocotyl, plant hormones treated in germinated mistletoe hypocotyls. *M. alba* was selected as the host in this hormone treatment test because inoculated mistletoe seeds on this tree showed low survival rates in the preceding test for screening host specificity of mistletoe. Seeds were taken from an approximately 20-year-old *Viscum album* var. *coloratum* (Kom.) Ohwi parasitizing on a *Quercus variabilis* tree on Mt. Jiri (Sannae-myeon, Namwon), at the end of February 2007 and stored at -2℃ for one month. After germination and hypocotyl elongation of inoculated mistletoe was complete, either 100 ppm or 200 ppm of IAA or kinetin hormones were applied between the mistletoe haustorium and the host bark used a brush.

Results

Seed germination of Korean mistletoe

To verify whether mistletoe seed germination is directly affected by temperature, an *in vitro* seed germination experiment was conducted. As shown in Fig. 1, seed germination rates of incubated mistletoe could be determined by observing hypocotyl emergence after about 20 days, and these hypocotyls grew up to 5 ㎜ over time (Fig. 1. B). The germination rate of mistletoe seeds at 15℃ was 92.8%, while the germination rate at 25℃ was 63.2%, it was shown a significant difference (Table 1; $p < 0.001$, F = 18.50).

Host specificity of Korean mistletoe seed

From the test to investigate the host-parasitic compatibility of Korean mistletoe with three species of *Rosaceae* and two species of *Fagaceae*, it was determined that seed germination, hypocotyl elongation, and haustorium plate formation of mistletoe were viable after one year on the branches of all of the tree species tested. As time went on, almost all seeds of inoculated mistletoe germinated and elongated their hypocotyls, and any differences caused by the five host species could not be detected. During the formation of mistletoe haustorium, any difference among the five trees was not shown. Regardless of the surface conditions of the host, mistletoe developmental steps from germination to haustorium formation successively progressed. As suggested by Tubeuf (1923), host plants might not provide mistletoe with any developmental influence during these periods. It was observed that new leaf emergence from the mistletoe hypocotyls at the spring of the second year

Table 1. Germination rate in vitro under the different temperatures

Temp. (℃)	Germination rate (%)
15	92.8
25	63.2

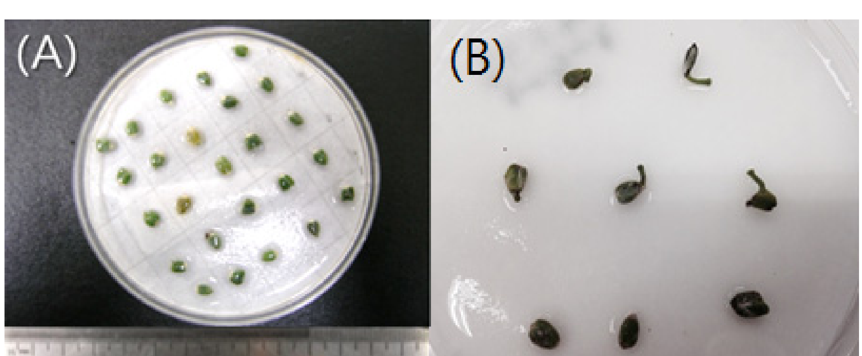

Fig. 1. Germination of Korean mistletoe seeds: seeds arranged on the petri dish (A) and hypocotyl elongated from seed (B).

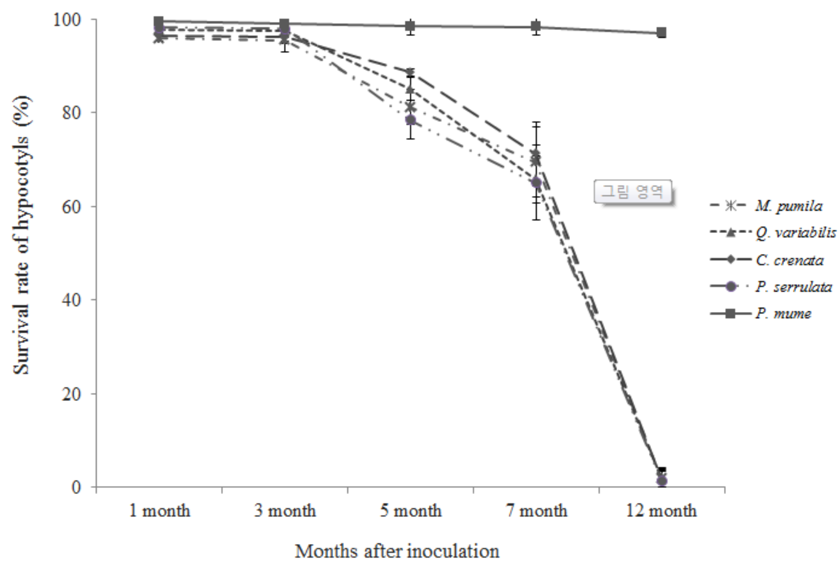

Fig. 2. Changes in hypocotyl survival on the host plant species at the next spring after inoculation of Korean mistletoe seeds. Vertical bars indicate standard error of the means (n = 3).

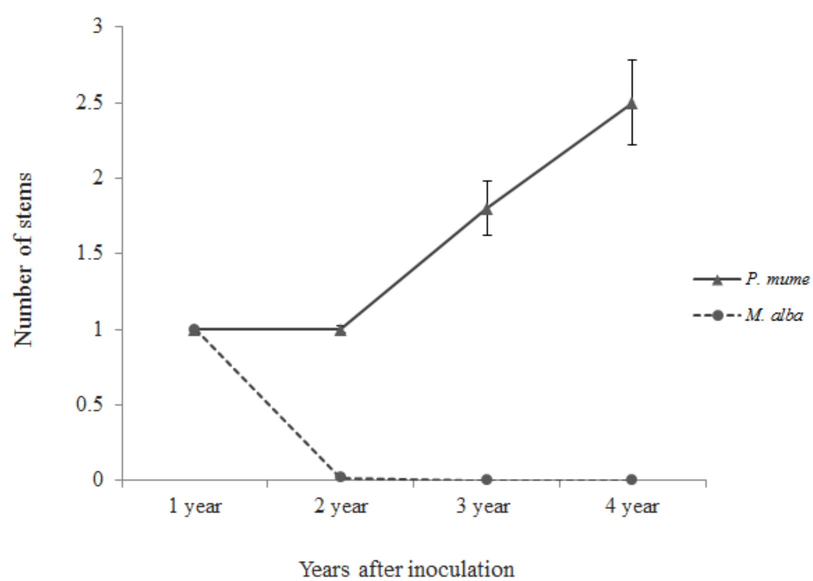

Fig. 3. Increase in the number of stems (hypocotyls at year 1) after the inoculation of Korean mistletoe seeds. Vertical bars indicate standard error of the means (n = 5).

was rare, as low as several percent, except on *P. mume* (Fig. 2). By the way, almost all the hypocotyls on the branches of *P. mume* survived to the autumn of the second year and elongated new leaves on their top at the spring of the third year, showing that the compatibility of Korean mistletoe with *P. mume* is surprisingly high, compared with the other tree species tested in this experiment (Fig. 4).

Field test for mistletoe seed inoculation

Table 2 describes the rates of inoculated seed germination, hypocotyl elongation, adhesive disk formation, and survival. Inoculated seed germination and adhesive disk formation were excellent on both *P. mume* and *M. alba,* and any difference between the two species was not detected. These results are consistent with the results of Tubeuf (1923), who suggested that the host surfaces in direct contact with mistletoe seeds

Table 2. Comparison of germination, hypocotyl elongation, adhesive disk formation, and stem survival between *P. mume* and *M. alba*

Time of investigation	First year of inoculation			Second year of inoculation	
Host	Germination rate	Elongated hypocotyl	Adhesive disk formation	Survival in spring	Survival in autumn
		(%)		(%)	
P. mume	99.2	99.2	98.4	98.4	97.6
M. alba	98.4	98.4	98.4	2.4	0

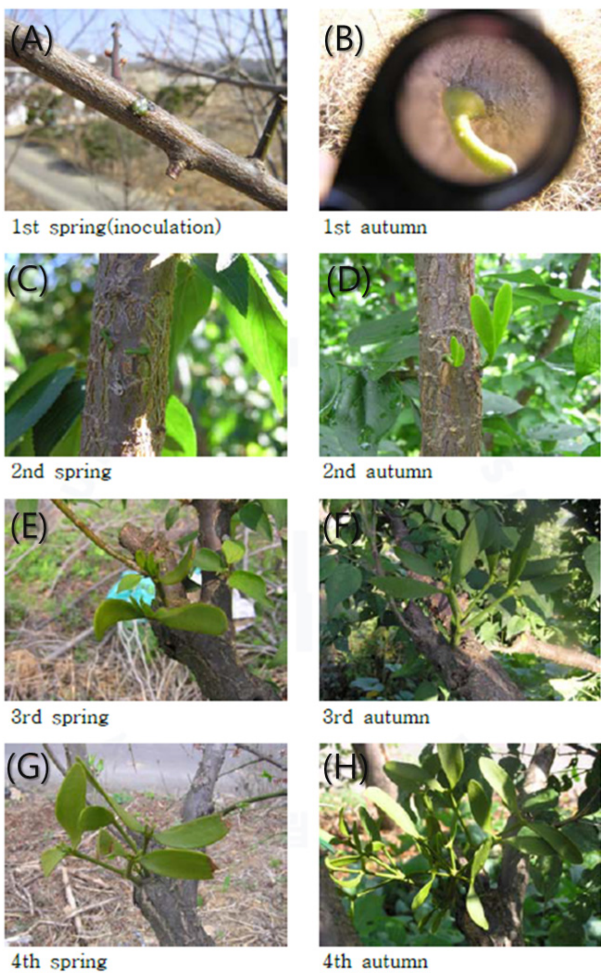

Fig. 4. Growing process of Korean mistletoe on the host *P. mume* after inoculation of seeds: seed germinated and elongated hypocotyl in 1st spring (A) and autumn (B); first leaves on the top of the hypocotyl and a new stem near the hypocotyl emerged in the 2nd spring (C) and autumn (D); a second set of leaves grew from a new node, which emerged on the first stem in the 3rd spring (E) and autumn (F); all stems grew vigorously in 4th spring (G) and autumn (H).

did not affect seed development. Moreover, this result implies that mistletoe does not have any specificity until hypocotyl elongation and disk formation, in addition to seed germination. While the survival rate of mistletoe seed inoculated on *P. mume* in the second year of inoculation was great (p < 0.001, F = 69.28), mistletoe seeds inoculated on *M. alba* showed extremely low survival rates, as low as 2.4%, and all of the surviving seeds were dead the following autumn. From these results, mistletoe is parasitic against *P. mume,* but does not have a parasitic relationship with *M. alba*.

Fig. 3 shows an increase in the number of nodes after artificial inoculation of mistletoe seeds for four years. The numbers of stems at the first year represent the number of newly growing hypocotyls, and those at subsequent years represent the number of nodes derived from newly grown hypocotyls with more than two leaves. While most mistletoe seeds inoculated on *M. alba* were dead in the autumn of the second year, as shown Table 2, on *P. mume* one to two (rarely three) hypocotyls developed from a single seed, and these hypocotyls started to penetrate the hosts' bark by forming endophytes. Also, the total number of stems increased, depending on the stems newly formed by the endophyte. These results are consistent with the schematic of the endophyte system suggested by Heide-Jørgensen (1989). Once again, after the third year, further increases in the number of stems were observed, and at the fourth year, the number of mistletoe stems that developed from one seed was more than two and a half.

Figures 4 and 5 shows the spring and autumn development of mistletoe seeds inoculated on *P. mume* and *M. alba*, respectively. For the first year, there were no differences between the inoculated seeds on the two plants investigated. Beginning in the second year, the number of mistletoe stems gradually declined and the inoculated seeds showed a 2.4% survival rate on *M. alba*. At the third year, the remaining mistletoe on *M. alba* was completely dead over the summer, whereas mistletoe on *P. mume* was vigorously growing.

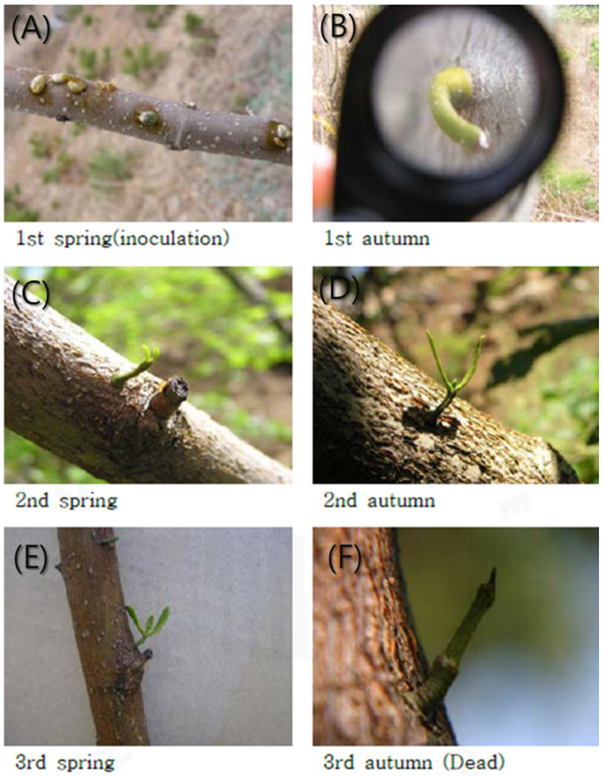

Fig. 5. Growing process of Korean mistletoe on the host *M. alba* after inoculation of seeds: seed germinated and elongated hypocotyl in 1st spring (A) and autumn (B); the first leaves on the top of hypocotyl emerged, but lost vigor in the 2nd spring (C) and autumn (D); first stem stopped growing in the 3rd spring (E) and finally died in the 3rd autumn (F).

Effects of hormone treatment for survival

To increase the survival rate of mistletoe, IAA and kinetin hormone were used to treat the inoculated seeds. Among the plant hormones, auxin and cytokine have been known to promote plant cell division, so we had expected that both hormones might have an effective impact on the formation of endophyte. In the spring of the year following mistletoe seed inoculation on *M. alba*, the survival rate was calculated by counting the newly development leaves of mistletoe seeds. Compared to the control without hormone treatment, however, treatments of either IAA or kinetin hormone did not increase the survival rate (Fig. 6).

Discussion

In the seed germination experiment designed for testing whether mistletoe germination is controlled by temperature at the beginning stage of seed germination, almost all of the seeds from Korean mistletoe grown on the *Quercus variabilis* germinated *in vitro* at the temperature of 15℃. However, the rate of seed germination at the temperature of 25℃ was 63 percentage. This result coincides with the suggestion by Lamont (1983) and suggests that seed germination is affected by temperature. In the testing of effect on the germination by the host plants, however, the initial germination rate was

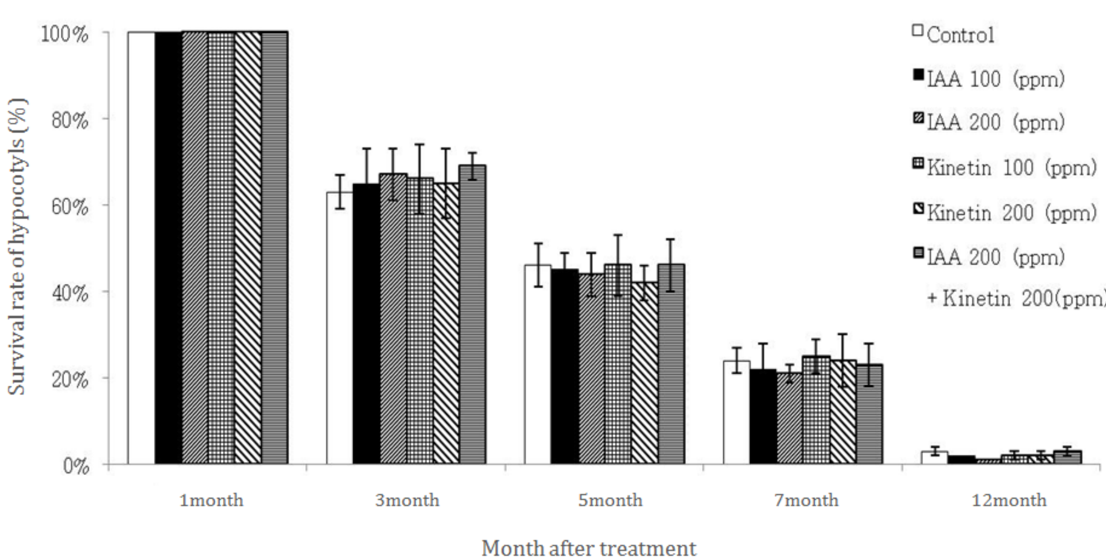

Fig. 6. Changes in hypocotyl survival rate after the inoculation of Korean mistletoe seeds on the host *M. alba*, according to the treatment of IAA and Kinetin hormones. Vertical bars indicate standard error of the means (n = 5).

excellent, no matter the species. This suggests that seed germination is not affected by the host species and is not the limiting factor for the early steps of artificial propagation. It is reported by Stopp (1961) that European mistletoe has dormancy and light affects mistletoe seed germination. Thus, scrupulous research on the relationship between dormancy and light is required. In addition, the collecting period and method for artificial cultivation of mistletoe have to be considered.

In the test for host specificity of mistletoe seed, in April of the second year after inoculation, the survival rates of inoculated mistletoe seeds were extremely low on all host species except on *P. mume*. This result is consistent with the artificial propagation experiment of mistletoe conducted by the Ministry of Agriculture (2000), which demonstrated that inoculated mistletoe seeds on all host species used in the tests showed extremely low survival rates of less than five percent. The survival rate of inoculated seeds was low on *M. pumila*. Surprisingly, the fact that mistletoe seeds inoculated on *P. mume* have very high survival rates is an exciting discovery; further in-depth study on the host specificity of Korean mistletoe is required.

Another trial to artificially cultivate Korean mistletoe by inoculation of seeds on *P. mume* and *M. alba* showed an extremely low survival rate on the branches of *M. alba* in the second year after seed inoculation and death after poor growth in the third year. On the other hand, there was a very high survival rate and continuously vigorous growth of mistletoe on the branches of *P. mume*, suggesting the possibility of artificial cultivation of Korean mistletoe with the selection of proper host species. In this new method, *P. mume* can be regarded as one of the best host plants for the artificial cultivation of mistletoe because it showed high parasitic affinity with Korean mistletoe.

In order to increase the survival rate on *M. alba*, inoculated mistletoe was treated with IAA and kinetin hormone. The treatments of IAA and kinetin at the stage of haustorium development could not reverse the low survival rate of Korean mistletoe on *M. alba*. It is possible that these hormones could not influence haustorium development and endophyte formation, or that the physical defense mechanisms of the host plant, *M. alba*, prevented endophyte invasion by parasitic mistletoe. This is supported by another study, which demonstrated that the treatment of IAA and kinetin has no effect on the seed germination of European mistletoe (Luther and Backer, 1986). These results suggest that mistletoe cell division and development at the period of germination or haustorium formation are not significantly affected by the treatment of exogenous auxins and cytokinins.

In this study, we suggest the possibility for artificial propagation of Korean mistletoe at a practical level, which may significantly contribute to the stable supply of materials for medicines and the conservation of natural resources.

Acknowledgement

This research was supported by ‘‘Establishment of Economic Technology for the Cultivation of Mistletoe and Development of Strategic Export Products Using Its Functional Compounds (Project No.: 313011-3),’’ Ministry of Agriculture, Food and Rural Affairs, South Korea.

References

Büssing, A., G.M. Stein, M. Wagner, B. Wagner, G. Schaller, U. Pfüller and M. Schietzel. 1999. Accidental cell death and gene ration of reactive oxygen intermediates in human lymphocytes induced by thionins from *Viscum album* L. Eur. J. Biochem. 262:79-87.

Büssing, A. (ED). 2000. Mistletoe: the genus *Viscum* (medicinal and asromatic plants－industrial profiles). Harwood Academic Press, Amsterdam, Netherlands.

Choi, J.Y. 2005. Lectin, ribosomal inactivating protein type II (RIP's), of Korean mistletoe (*Viscum album* var. *coloratum*) from different host-species. MS Thesis, Dongkuk Univ., Korea (in Korean).

Hariri, E.B. and C. Andary. 1991. Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.) Protoplasma 162:20-26.

Heide-Jørgensen, H.S. 1989. Development and ultrastructure of the haustorium of *Viscum Minimum*. Can. J. Bot. 67(4): 1161-1173.

Jang, C.S. 1997. Biochemical characterization and pharmacological effect of lectin from *Viscum album*. Ph.D. Thesis, Keimyung Univ., Korea (in Korean).

Jun, Y.H. 2004 Study on the immunostimulant activity of the extract of Korean mistletoe fruits to activate macrophages in mice. MS Thesis, Konyang Univ., Korea (in Korean).

Jung, B.Y. 1999. Study of biological activity components of Korean and European mistletoe. MS Thesis, Seoul Women's Univ., Seoul, Korea (in Korean).

Jung, J.H. 2005. Adjuvant effect of Korean mistletoe lectins on the induction of systemic and mucosal immunity by intranasal immunization. MS Thesis, Handong Univ., Korea (in Korean).

Kim, S.H. 1997. Isolation and characterization of lectin using the Sepharose 4B column from Korean mistletoe (*Viscum album coloratum*). MS Thesis, Kunkook Univ., Korea (in Korean).

Kim, T.J. 1996. Korean resources plants I: *In* Kim, T.J. (ed.), Korean Resources Plants, Seoul National University Press, Seoul, Korea. pp. 140-141 (in Korean).

Lamont, B. 1983. Germination of mistletoes: *In* Calder, M. and P. Bernhardt (eds.), The Biology of Mistletoes. Academic Press, Sydney, Australia. pp. 129-143.

Lee, B.D. 2009. Ecological environment of native habitats and host plant in mistletoe (*Viscum album* var. *coloratum*). Korean J. Plant Res. 22(5):389-393 (in Korean).

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(4):287-292 (in Korean).

Lee, G.P. 2003. Identification and purification of lectin from induced callus in Korean mistletoe (*Viscum album* var. *coloratumn*). MS Thesis, Dongkuk Univ., Korea (in Korean).

Lee, S.J. 2002. The screening of biological activities for Korean *Viscum* sp. classified by host trees. MS Thesis, Kangwon Natl. Univ., Korea (in Korean).

Lee, T.B. 1985. Illustrated flora of Korea. 3rd ed., Hyangmoonsa, Seoul, Korea (in Korean).

Luther, P. and H. Becker. 1986. Die Mistel: Botanik, Lektine, medizinische Anwendung. VEB Verlag Volk und Gesundheit, Leimen, Germany (in German).

Lyu, S.Y., S.M. Park, B.Y. Choung and W.B. Park. 2000. Comparative study of Korean (*Viscum album* var. *coloratum*) and European mistletoes (*Viscum album*). Arch. Pharm. Res. 23(6):592-598.

Machaidze, G., K. Urech and D. Mikeladze. 1996. Comparative study on the modulatory effect of plant lectins and viscotoxins on the *N*-methyl-d-aspartate receptor in synaptic plasma membranes: *In* Driessche, E.V., P. Rougé, S. Beeckmans and T.C. Bog-Hansen (eds.), Lectins: Biology, Biochemistry, Clin. Biochem. Vol. 11, Textop, Hellerup, Denmark. pp. 265-272.

Martin, L.L., C.R. Friedman and R. G. Smith. (2012). Callus culture of *Arceuthobium americanoum* Nutt. ex Englem. and explant response to varying auxin and cytokine concentrations. Plant Tissue Culture and Biotechnology 21(1):1-10.

Ministry of Agriculture. 2000. Technical development for the utilization and cultivation of Korean mistletoe. Ministry of Agr., Gwacheon, Korea (in Korean).

Ministry of Agriculture. 2007. Studies on genetic property and useful substances of *Viscum album* and *Ulmus davidiana* var. *japonica* for the development of functional cosmetics. Ministry of Agr., Gwacheon, Korea (in Korean).

Park, S.M. 1999. Isolation and characterization of biological activity high molecular substances from *Viscum album* var. *coloratum*. MS Thesis, Seoul Women's Univ., Korea (in Korean).

Stopp, F. 1961. Unsere Misteln, A. Ziemsen, Wittenberg, Germany (in German).

Sung, G. 2005. Antiangiogenic mechanisms of Korean mistletoe lectin. MS Thesis, Sungkyunkwan Univ., Korea (in Korean).

Tubeuf, C. 1923. Monographie der mistel. München und Berlin, Oldenburg, Germany (in German).

Visser, J. 1981. South African parasitic flowering plants. 1st ed, Juta, Cape Town. South Africa.

Yoon, T.J. 1997. Studies on the biological activities of lectin purified from Korean mistletoe (*Viscum album coloratum*) extracts and its characterization. Ph.D. Thesis, Kunkook Univ., Korea (in Korean).

(Received 3 June 2015 ; Revised 4 November 2015 ; Accepted 12 November 2015)