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Original Research Article

Host Plant Management Techniques for the Cultivation of *Viscum album* var. *coloratum* (Kom.) Ohwi

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Abstract - Research has found that the management of the host plant is essential to mistletoe cultivation. A Trunk injection test on the host plant that contained a mixture of indole-3-butryc acid (IBA) and liquid fertilizer was conducted with respect to the improvement of the one year survival rate of mistletoe. As a result, the trunk injection experiments showed the effect of the IBA and liquid fertilizer mixture in all treatments. This mixture was effective to increase the survival rate of mistletoe by 20% with the IBA at 100 ㎎/L and Hyponex at 100 ㎎/L. The examination proved that the host plant fertilizer effect was the most effective treatment for organic fertilizer with 60% added NPK (4-2-1). Its effects were higher compared to the control at the length and diameter of one-years-old branches in the host plant, even when the parasitic mistletoe improved its growth in length, diameter, and number of branches. Comparing the control and host plant fertilizer, the latter was the most effective way to process 20 ㎏ per a treatment effect in the experimental site and to process at any time after the inoculation. This treatment is effective to improve the growth of mistletoe by watering the host plant three times per week. Therefore, the management of the host plant is an essential element in the successful cultivation of mistletoe, not only to supply fertilizer and plant hormones to the host plant in the initial inoculation time but also to provide organic fertilizer and irrigation for the host plant.

Key words - *Viscum album* var. *coloratum*, Inoculation, Survival rate, Fertilizer effect, Host plant, Cultivation techniques

Introduction

Mistletoe is a photosynthetic hemiparasite that attaches to the branches of a host plant. About 40 genera and 1,500 species are distributed in tropical and temperate regions, apart from cold regions (Visser, 1981). The genus of *Viscum* contains about 30 species distributed across Asia, Africa, Europe, and Australia (Kim, 1996). Korean mistletoe (*V. album* var. *coloratum* [Kom.] Ohwi) is distributed in the coldest place in South Korea. Host plants in Korea include *Quercus* spp., *Celtis sinensis*, *Q. dentata*, *Betula pyatyphylla* var. *japonica*, *Salix koreensis*, *Alnus japonica*, and *Castanea crenata* var. *dulcis* (Lee, 2009). The main distribution area is above sea level (0 ~ 1,200 m), which is not affected by the surface conditions (Lee, 2009).

Research on national and international varieties of mistletoe mainly focuses on the plant's medical effects and components. Mistletoe contains the major anticancer components of lectin and viscotoxin (Bloksma *et al*., 1979; Franz *et al*., 1981; Khwaja *et al*., 1986; Ribéreau-Gayon *et al*., 1986; Doser *et al*., 1989; Hajto *et al*., 1990; Kuttan and Kuttan, 1992; Schink, 1997; Park *et al*., 2003; Hwang *et al*., 2003). The medicinal effects of mistletoe include elevated immune function, cancer cell inhibition, lowered blood pressure, and joint laxity. In particular, mistletoe has a therapeutic effect for hypertension, angina, diabetes, stroke, paralysis of hands and feet, and back pain (Choo, 2001). Currently, because there are no mistletoe-based injections developed in Korea, the use of injections contained mistletoe anticancer components are depended on importation from Europe (Kim *et al*. 2013). This mistletoe extract is imported and marketed in South Korea and is being utilized in hospitals. Drug discovery research using Korean mistletoe is now in progress (Jung, 2000).

Recently, the use of Korean mistletoe has caused problems, such as damage to the environment incurred by illegal collection and host plant lumbering. Even if there is a problem with the supply of raw materials, mistletoe is being used to

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develop medicines or health foods (Lee, 2009). From 1997 to 2000, the Ministry of Agriculture carried out ecological surveys and studies for artificial cultivation technology of growing mistletoe (Ministry of Agriculture and Forestry, 2000). Since then artificial cultivation technology and tissue culture experiments have been conducted (Ministry of Agriculture and Forestry, 2007), but this research is lacking basic information about the artificial cultivation of the plant. Recently, our research group has carried out a study of mistletoe cultivated with plum trees as the host plant (Lee, 2010). This research has progressed to mass propagation and commercialization. Based on the results of artificial cultivation, host plant growth management is essential. Therefore, using the host plant cultivation management is expected to be able to control the growth of parasitic plant.

Through these studies, we hope to provide the basic data required for the artificial cultivation technology for mistletoe.

Materials and Methods

Sample preparation and test site

As an experimental material, mistletoe (*V. album* var. *coloratum* [Kom.] Ohwi) is a parasitic on oriental oak (*Q. variabilis*). Mistletoe seeds were collected in February 2013 and stored in a -2℃ low temperature refrigerator for 15 days. Then, in March 2013, the pericarp of the fruit was removed and the viscosity of the sarcocarp was inoculated on five years-old plum trees (*Prunus mume*). The design of the experiment was carried out for five plum trees with a test port and three replicates. The two years-old branches of the plum trees were inoculated with five pairs of mistletoe. Observing the germination and growth of mistletoe for the first year, a test site was created in the sprouts of normal mistletoe leaves in the spring of the following year. In the same way, the host plant management experiment was carried out with plum trees of two and five years. All tests observed the growth of the host plant and any changes of mistletoe growth.

A two hectare natural deciduous forest was set up for the test site in the Namyangju Gyeonggi Province, Korea. The characteristics of natural forest hardwoods, including species composition, consisted of *Q. aliena* (45%), *Q. mongolica* (30%), *P. koraiensis* and *B. davurica* (Table 1). The soil characteristics included acidity (pH 5), loamy sand and sandy loam (typical of forests) and high organic content in the soil horizon. Hardwood lumber-cutting areas were selected as test areas (Table 2).

Trunk injection test for growth regulation after mistletoe inoculation

Mistletoe was inoculated into five years-old plum trees, and the experiment was conducted to increase the survival rate by trunk injection in the host plant. By this method, the inoculated mistletoe was injected with the IBA (Sigma, USA) and liquid fertilizer (Hyponex, Japan) mixture. Trunk injection solutions were composed of 50 ppm to 100 ㎎/LIBA and 50 ㎎/L and 100 ㎎/L liquid fertilizer in a 5% glucose solution (1,000

Table 1. Vegetation characteristics of field test area in Gyeonggi Province

Area	Forest condition	Species	RD[z] (%)	RC[y] (%)	IV[x] (%)
Gyeonggi-do Namyangju	Nature	*Quercus aliena*	45	45	45
		Quercus mongolica	30	30	30
		Betula davurica	5	5	5
		Cornus controversa	1	1	1
		Pinus koraiensis	1	1	1
		Symplocos chinensis for. Pilos	5	1	3
		Lindera obtusiloba	5	2	3.5
		Rhus tricocarpa	5	2	3.5
		Calamagrostis arundinacea	1	3	2
		Solidago virgaurea subsp. asiatica	1	1	1

[z]R.D (relative density) = total numbers of some plant/total number of occurrence×100.
[y]R.C (relative coverage) = area of some plant/total area×100.
[x]I.V (importance value) = R.D + R.C/2.

Table 2. Soil characteristics of field test site in Gyeonggi Province

Item	Unit	Analysis	
		A horizon (0.0~10 ㎝)	B horizon (10.0~40 ㎝)
pH(1:5)	-	6.15	6.56
EC(1:5)	(dS/m)	0.21	0.17
Organic	(g/㎏)	52.3	43.5
Phosphate	(㎎/㎏)	72	51
EX. Ca	(cmol(+)/㎏)	2.56	3.63
EX. K	(cmol(+)/㎏)	0.65	0.39
EX. Mg	(cmol(+)/㎏)	2.76	3.05
EX. Na	(cmol(+)/㎏)	0.34	0.29
Texture	-	Loamy sand	Sandy loam
Sand	(%)	72.2	55.1
Silt	(%)	19.8	38.5
Clay	(%)	3.9	6.2

㎖). A drill was used to put a hole in the bottom of the mistletoe branches, and the host plant was injected with an aqueous solution after one month, since the mistletoe inoculation success rate is higher when the host plant has characteristics of vigorous growth. Experiments were carried out to promote the host plant's growth by the IBA and liquid fertilizer and to achieve a one year survival rate for the mistletoe.

Test for moisture management in the mistletoe's host plant management

Irrigation management tests were carried out the growth of the host plant and mistletoe could be controlled. For irrigation water management, mechanical spreaders (Deadong, Korea) were used to spray the water supply onto the mistletoe's host plants. Except on rainy days, regular watering was performed from March to September of 2013. The characteristics of the study area also made use of reclaimed areas, which represents the mountain slope and soil characteristics of the mountainous area. Therefore, in the cultivation of plants, it was determined that water management has an essential influence on the growth of the host plant and the mistletoe.

Results and Discussion

Trunk injection test for growth regulation after mistletoe inoculation

Experimental investigation of the results and the residual effects of the control test, which used trunk injection for pine wilt (*Bursaphelenchus xylophilus*), was performed (Takai *et al*., 2003). In the study of pine-wilt control and the residual effects of trunk injection for black pine (*P. thunbergii*) and Korean pine (*P. koraiensis*), the effect was sustained at approximately 30-days (Lee *et al*., 2009). Therefore, we tested the effects of injecting a liquid fertilizer and plant hormones directly in to the tree on the growth of the plum tree-host plants, and we also tested the effects on the mistletoe's one-year survival rate.

The mistletoe germinated in the spring and adsorbed haustorium on the bark of the plum trees; it started to grow using haustorium when water and nutrients were supplied. Typically, if haustorium development does not attain the phloem, xylem, and vascular tissue of the host plant, the mistletoe withers away within about six months (Lee, 2010). Mistletoe seeds that inoculated in the spring have a higher initial germination period to form a haustorium. However, the one-year survival rate is low for these seeds. Taking these characteristics into consideration (Improving the survival rate after inoculation) is an important factor in the growth of mistletoe. This study was performed to test for ways to increase the survival rate after one year. Auxin is a hormone involved in the elongation of plant cells. Auxin and liquid fertilizer with water from the host plant were used to increase the initial growth of the mistletoe by supplying sufficient inorganic nutrients and plant hormones.

As a result, the treatment of mistletoe and an increase of the one-year survival rate phenomenon were observed. In particular,

the IBA 100 ㎎/L and Hyponex 100 ㎎/L treatments for the one-year survival rate shows the effect of keeping the level of 40%. This represents a 20% improvement of the survival rate compared to the untreated plants' survival rate of less than 20% (Fig. 3). After inoculating the mistletoe in the early growing season, the survival rate is expected to increase through the direct hormonal and nutrient supply of a trunk injection.

Testing the effects of organic matter and fertilizer for mistletoe host plant management

It is known that, when supplied with moisture and nutrients from the host plant, the growth of parasitic plants can be maintained. In particular, Lee (2009, 2010) shows that the health of the host plant influences the growth and survival of mistletoe. The Ministry of Agriculture and Forestry (2007) reported that mistletoe inhibits the growth of the host plant. Therefore, tests were carried out to determine whether the growth characteristics of mistletoe promote the growth of the host plant and whether mistletoe can be expected to affect growth promotion.

Organic fertilizer, 60% and 40%, was mixed to promote the growth of plum trees, and the complex fertilizer was mixed with 5% of the NPK (4-2-1) and NPK (12-5-7). This was to increase the effectiveness of the research objectives by utilizing existing products.

The results of the high content of organic matter and nitrogenous fertilizer in the experiment site were investigated by examining the branch length (25 ㎝) and diameter growth (2.8 ㎝) of one-year-old host plants (Table 3); the low content of organic matter and nitrogenous fertilizer in the experiment site was investigated by examining the branch length (23 ㎝) and diameter growth (2.3 ㎝) in one-year-old host plants. Also, the growth of the mistletoe was investigated by assessing branch length and diameter growth. Compared to the low and high concentration fertilizer treatments, the length of the mistletoe increased from 6.2 to 6.5 ㎜, and the diameter of the mistletoe increased from 2.4 to 2.6 ㎜, confirming the growth-promoting effect on the mistletoe. The quantity of fertilizer per unit test was compared to the treatments with organic fertilizer at 10 and 20 ㎏; the branch length of the host plant increased from 25 to 31 ㎝. For the growth of the

Table 3. Treatment of organic fertilizer for the growth regulation of mistletoe

Treatment	Amount (a/㎏)	1 year Branch			Mistletoe		
		Length (㎝)	Diameter (㎝)	Number (ea)	Length (㎜)	Diameter (㎜)	Number (ea)
control		15.8±4.2	2.5±2.2	12.7±5.8	6.0±1.4	1.9±0.6	1.5±1.0
60%(4-1-1)	10	25.4±7.6	2.8±6.2	22.3±7.9	6.5±1.6	2.6±0.4	1.5±1.0
40%(12-5-7)	10	23.5±8.4	2.3±7.2	21.6±3.2	6.2±1.3	2.4±0.5	1.5±1.0
60%(4-1-1)	20	31.3±6.7	2.8±4.9	29.3±9.7	7.5±1.6	2.8±0.7	2.5±1.0
40%(12-5-7)	20	24.2±8.4	2.6±4.5	25.8±6.6	7.0±1.3	2.6±0.3	2.5±1.0

Table 4. Treatment of organic fertilizer for the growth regulation of inoculation 2 and 5 years mistletoe

Treatment	Amount (a/㎏)	1 year Branch			Mistletoe		
		Length (㎝)	Diameter (㎝)	Number (ea)	Length (㎜)	Diameter (㎜)	Number (ea)
Inoculation 2 years	-	7.8±4.2	2.4±2.1	51±7	6.0±1.4	1.9±0.6	1.5±1.0
Inoculation 2 years	10	7.7±3.4	2.8±1.1	53±6	6.5±1.6	2.6±0.4	1.5±1.0
Inoculation 2 years	20	8.3±3.2	3.8±2.2	50±7	7.5±1.6	2.8±0.7	2.5±1.0
Inoculation 5 years	-	3.7±1.2	2.2±1.7	152±12	75.9±1.6	42.5±0.6	8.5±1.0
Inoculation 5 years	10	4.2±3.2	2.4±2.9	148±32	76.3±1.7	46.7±0.4	9.0±1.5
Inoculation 5 years	20	5.1±4.3	2.6±2.5	158±40	89.3±1.6	50.5±0.8	9.5±1.5

Table 5. Irrigation tests for the growth regulation of mistletoe

Treatment	1 year Branch			Mistletoe		
	Length (㎝)	Diameter (㎝)	Number (ea)	Length (㎜)	Diameter (㎜)	Number (ea)
Control	15.8±4.2	2.5±2.2	12.7±5.8	6.0±1.4	1.9±0.6	1.5±1.0
Once a week	20.3±4.4	2.3±6.4	17.3±6.2	6.2±1.7	2.1±0.5	1.5±1.0
Three times a week	22.3±6.4	2.6±3.1	26.7±7.9	6.5±1.4	2.5±0.6	1.5±1.0

Fig. 1. Appearance of mistletoe growth on plum tree.

Fig. 2. Appearance of trunk injection.

mistletoe, the length of the branches increased from 6.5 to 7.5 ㎜, and the number of branches increased from 1.5 to 2.5.

In these comparative experiments of fertilization's effect on two-year-old and five-year-old plum trees with parasitic mistletoe, using 20 ㎏ of fertilizer was found to be more effective than using a 10 ㎏ site. So, the growth of the one-year-old branches of the host plants increased by about 1 ㎝ in length and diameter; similarly, the length of the mistletoe was improved by about 1 ㎜ in the two-year-old mistletoe and

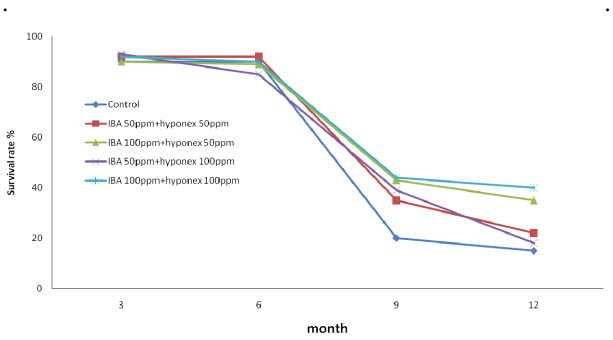

Fig. 3. Improved survival rate after inoculation test of mistletoe.

10 ㎜ in the five-year-old mistletoe (Table 5).

The fertilization experiments for host plant management were found to be advantageous for the stable growth of five-year-old to two-year-old mistletoe after inoculation, and processing the 20 ㎏ per site was more effective in both treatment groups.

Test for the moisture management of mistletoe host plant management

Areas of growth for mistletoe cultivation and host plants are mainly located in reclaimed land for forests, and such land is characterized by the severe leaching of water and nutrients from the soil. These lands still share many characteristics with mountainous and barren slopes. Also, the climate characteristics of the Korean peninsula have high rainfall during the rainy season, and the annual rainfall distribution is not uniform. Therefore, the cultivation of plum trees inoculated with mistletoe is essential in water management of the cultivation site. Water stress on the host plant affects the growth of the parasitic plant; accordingly, we mentioned the need for the management of the host plants (Lee, 2010). Considering these characteristics, moisture management research for efficient cultivation of mistletoe was performed.

Branches of 2 years inoculation

Measurement of 2 years mistletoe diameter

Measurement of 2 years mistletoe length

Branches of 5 year inoculation

Measurement of 5 years mistletoe diameter

Measurement of 5 years mistletoe length

Vigorous growth of host plant (increased 2.5folds of mistletoe number)

Vigorous growth of host plant (deformation of branch form from Y to W)

Vigorous growth of host plant (vigorous growth of mistletoe)

Fig. 4. Measurements of mistletoe cultivation experimental site.

As compared to non-treatment, moisture treatment was found to affect the branch length and number of one-year-old plum trees. For irrigation conducted three times a week compared to once a week, the branch length increased from 20 to 22 ㎝, branch diameter increased from 2.3 to 2.5 ㎝, and the numbers of branches increased from 17 to 26 for one-year-old trees plum. This could indicate that water management promotes the growth of host plants on all sides. It even affected the growth of the mistletoe, increasing the length of branches by about 0.5 ㎜ and the diameter by about 0.5 ㎜. Therefore, water management with host plants is known to be an important factor in the cultivation of mistletoe.

Conclusion

Through the study of the artificial cultivation of mistletoe (*V. album* var. *coloratum* [Kom.] Ohwi), the vitality of host plants was found to have an impact on the growth of mistletoe. We can draw several conclusions from this. First, a nutrient and hormone supply test was conducted by trunk injection to improve the mistletoe's survival rate one year after the host plant inoculation. As a result, both the treatment of the mistletoe and an increase of the one-year survival rate phenomenon were observed; in particular, the IBA 100 ㎎/L and Hyponex 100 ㎎/L treatments for the one-year survival

rate shows the effect of keeping the level of 40%. This represents an improved survival rate of 20% compared to the untreated plants' survival rate of less than 20% (Fig. 3). After the inoculation of the mistletoe in the early growing season, the survival rate is expected to increase through the direct hormonal and nutrient supply by trunk injection.

Second, the host plant growth-promoting experiments were carried out through the promotion of mistletoe as way to test the effects of organic matter and fertilizer for mistletoe host plant management. The results of the high content of organic matter and nitrogenous fertilizer-treated experiment site were investigated by examining the branch length (25 ㎝) and diameter growth (2.8 ㎝) of one-year-old host plants (Table 3); the low content of organic matter and nitrogenous fertilizer treated experiment site was investigated by examining the branch length (23 ㎝) and diameter growth (2.3 ㎝) in one-year-old host plants. Also, the growth of the mistletoe was investigated by assessing branch length and diameter growth. Compared to the low and high concentration fertilizer treatments, the length of the mistletoe increased from 6.2 ㎜ to 6.5 ㎜, and the diameter of the mistletoe increased from 2.4 ㎜ to 2.6 ㎜, confirming the growth-promoting effect on the mistletoe. The quantity of fertilizer per unit test was compared to the treatments with organic fertilizer at 10 and 20 kg; the branch length of the host plant increased from 25 to 31 cm. For the growth of the mistletoe, the length of the branches increased from 6.5 to 7.5 ㎜, and the number of branches increased from 1.5 to 2.5.

In these comparative experiments of fertilization's effect on two-year-old and five-year-old plum trees with parasitic mistletoe, using 20 kg was found to be more effective than using a 10 kg site So, the growth of the one-year-old branches of the host plants increased by about one cm, even if the length of the mistletoe improved by about one ㎜ in the two-year-old mistletoe and by about 10 mm in the five-year-old mistletoe (Table 4). The fertilization experiments for host plant management were found to be advantageous for the stable growth of five-year-old to two-year-old mistletoe after inoculation, and processing the 20 kg per site was more effective in both treatment groups.

Finally, these growth-promoting experiments for host plants were carried out through moisture management. As compared to non-treatment, moisture treatment was found to affect the branch length and number of one-year-old plum trees. For irrigation conducted three times a week compared to once a week, the branch length increased from 20 to 22 ㎝, branch diameter increased from 2.3 to 2.5 ㎝, and the number of branches increased from 17 to 26 for one-year-old trees plum. This could indicate that water management promotes the growth of host plants on all sides. It even affected the growth of the mistletoe, increasing the length of branches by about 0.5 mm and the diameter by about 0.5 mm. Therefore, inoculating the mistletoe in the early growing season is expected to be able to increase the survival rate of mistletoe through direct hormone and nutrient supply by a trunk injection. In the fertilization experiments for host plant management, processing 20 kg per site was found to be advantageous. It was also more effective in both treatment groups, from the stable growth of five-year-old to two-year-old mistletoe after inoculation, and the water management in the cultivation of mistletoe with host plants was also found to be a very important factor.

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