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Effects of Plug Cell Trays, Soil and Shading Rates on Seed Germination and Seedling Growth Characteristics of *Hippophae rhamnoides* L.

Songhee Lee[1], Wonwoo Cho[2], Romika Chandra[1], Jiwon Han[1] and Hoduck Kang[1,*]
[1]*Department of Biological and Environmental Science, Dongguk University, Goyang 10326, Republic of Korea*
[2]*Department of Forest Bioresources, National Institute of Forest Science, Suwon 16631, Republic of Korea*

Abstract

In this study, basic data with respect to the introduction of *Hipphophae rhamnoides* L. and its cultivation in Korea could be obtained. According to the size of the plug cell tray, Chinese origin's rate of seed germination was relatively high in 128 plug cell tray, and growth was vibrant in 50 plug cell tray. The germination and growth of Russian origin seeds showed that they were relatively effective in 50 plug cell tray and with respect to soil environment, TKS-2 soil with untreated shading relatively promoted both germination and growth for Chinese origin, the rate of germination was high in bed soil for horticulture and growth result was good in TKS-2 in the case of Russian origin. It was confirmed that the germination rate of Chinese origin *H. rahmnoides* L. was highest in untreated shading and the shoot growth was vibrant in 70% shading while the growth in roots was vibrant in the untreated shading. In the Russian origin, *H. rhamnoides* L. the germination rate in 30% and 70% shading was about 50% which was higher than that in the untreated shading and general growth was vibrant in 30% shading.

Key Words: KNO_3, pre-treatment, propagation, sea buckthorn, seedlings

Introduction

Hipphophae rhamnoides L. (Sea buckthorn) a deciduous broad-leaved tree belonging to Elaeagnacae, is distributed in Europe, China, Mongolia, and Russia and there are 6 species with 12 subspecies in the world (Li and Schroeder 1996; Heinäaho et al. 2006). Sea buckthorn grows up to 2-4 m high and is dioecious with separate male and female plants, has a characteristic of long branches with brown thorns and silvery grey lanceolate leaves growing narrowly alternate and its fruits are harvested around October. Sexual reproduction and asexual propagation are possible and grow in dry and semi-dry regions are possible. Trees belonging to the Elaeagnacae family have a feature to grow well in various climatic zones (Montpetit and Lalonde 1988). Sea buckthorn can also grow in -40°C and is very hardy and strong in resisting salt and aridity.

Trees of Elaegnacae including the sea buckthorn have difficulties in seed germination due to physiological dormancy of inner parts of the seeds and according to Zhapakova and Vernik (1986), it has been reported that seeds of sea buckthorn showed about 80% difference in germinating rate according to whether seed dormancy had been broken or not. Especially, the studies related with *in vivo* propagation of the seeds of sea buckthorn have been reported with respect to breaking dormancy of sea buck-

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Corresponding author: Hoduck Kang
Department of Biological and Environmental Science, Dongguk University, Goyang 10326, Republic of Korea
Tel: 82-31-961-5121, Fax: 82-31-961-5108, E-mail: hdk0225@dongguk.edu

thorn seeds mainly through pre-treatment methods (Pearson and Rogers 1962; Sankhyan et al. 2005; Olmez 2011; Liu et al. 2017; Olmez and Olcum 2017).

In addition, the local sea buckthorn studies on physicochemical effectiveness analysis and physiological vitality (Kim 2010; Kim et al. 2010; Park et al. 2010) have been actively performed, while the study on cultivation is not enough. A cultivation study on cutting propagation of sea buckthorn and seed germination was performed by Choi (2012). According to the increase of local interest in sea buckthorn farmers cultivating sea buckthorn has increased in numbers, however, an insensible introduction and lack of information and technology in the propagation of seeds and raising of seedlings cause difficulties in the cultivating process.

Furthermore, local seed germination tests are mainly performed *in vitro*, hence making it difficult to apply it in the field and farms where climate variations are high (Kang et al. 2004). The study related with *in vivo* culture of sea buckthorn studies has been actively performed with respect to the breaking of seed dormancy mainly through seed pre-treatment. Mainly *in vitro* studies have been performed where studies focus on soil and shading which are insufficient when cultivating sea buckthorn in actual farms.

Therefore, in order to secure high value-added materials, the useful genetic source of sea buckthorn introduced from China and Russia, and to increase the spread of this dioecious species. This study was performed focusing on the effects of the size of plug cell tray, shading methods and soil compositions and how these influence seed germination and growth of sea buckthorns.

Materials and Methods

Materials

Testing materials for this study, seeds of Russian origin sea buckthorn had been received from Geoecology Institute of Mongolian Academy of Science and those of Chinese origin had been received from Institute of Prevention of Yellow Dust and Desertification, Inner Mongolia, China and had been kept in 4°C before they were used. Seeds were harvested the year before the experiment was conducted.

Effect of plug cell tray, soil condition and shading treatment

To determine increased efficiency of cultivating sea buckthorn seeds by origin, the seeds were dipped in 100 ppm KNO_3 for 24 hours (Airi et al. 2009). In order to investigate the effects of the plug cell tray and its influence on seed germination, the seeds were sown in the tray after being filled up with TKS-2 (Floragard, Germany) on 50, 72, 128, 200, 288 trays (30 cm×60 cm×6 cm). For the effects of soil conditions, the seeds were sown in the tray after being filled with TKS-2, bed soil for horticulture soil-cement (Baroco, Seoul Bio. Co. Ltd) and sandy soil. The effects of shading treatment on sea buckthorn germination and seedling were performed in 128 plug cell trays filled with TKS-2 and were raised in separately treated plot, without shade (0%) with 30%, and 70% shading.

Three seeds per cell in each 50, 72, 128, 200, 288 plug cell tray were repeated three times. After planting the seeds, the trays were watered every day. Four weeks after sowing the seeds, germination rates were measured with the interval of a week to 6 weeks, after which germination rate determination was completed. Growth of plantlets after germination, plant length (cm), leaf length (cm), leaf width, No. of leaves, root length (cm), and fresh weights [shoot and root (g)] were measured during the 4^{th} and 8^{th} week. Germination and growth tests had been conducted in a greenhouse located in Gyeryu-ri Pocheon-si, Gyeonggi-do, Republic of Korea (168 m above sea level).

Statistical analysis

The data from this experiment are presented as means± standard deviation obtained from three or more repetitions. An One-way analysis of variance (ANOVA) was performed in order to determine variation between groups. The differences between these groups were compared using Duncan's multiple range test at 5% significance level. All statistical calculations were performed using SPSS Version 22 (IBM Co., USA).

Results

Effect of plug cell tray on seed germination and seedling growth

The seeds of Chinese origin showed about 30% germination rate, the highest observed in 128 plug cell tray (Fig. 1) and it was confirmed that the germination rate was high in the order of 50, 200, 288, and 72 plug cell tray. The growth of Chinese origin plantlets was vibrant in 50 plug cell tray with plant length (7.8 cm), leaf length (4.0 cm), root length (8.8 cm), leaf width (0.7 cm), No. of leaves (8), new shoot diameter (0.5 mm) showing relatively more vibrant growth than in 128 plug cell tray (Table 1, Fig. 2).

The highest germination rate in Russian origin seeds was confirmed in 50 plug cell tray at about 45% in 50 plug cell tray showing the highest germination rate, and the germi-

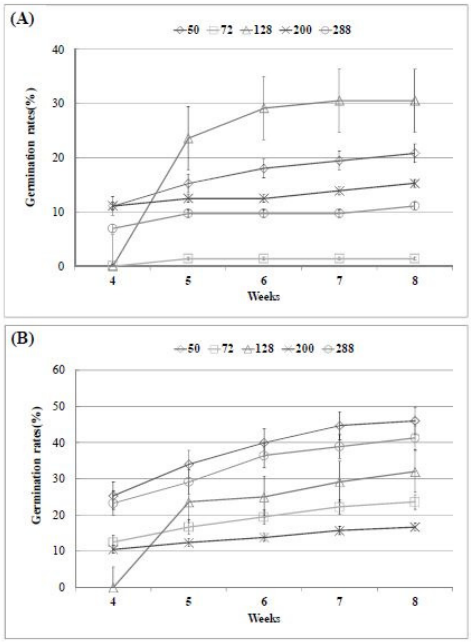

Fig. 1. Effect of No. of cells/tray of seed for germination rates (%) of different sources of *H. rhamnoides* L. (A) China and (B) Russia.

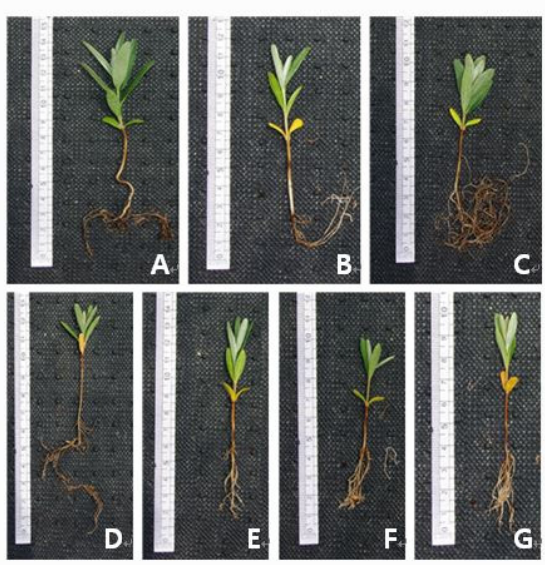

Fig. 2. Effect of No. of cells/tray of different sources of *H. rhamnoides* L. (A) China, 50 plug cell tray, (B) China, 128 plug cell tray, (C) Russia, 50 plug cell tray, (D) Russia, 72 plug cell tray, (E) Russia, 128 plug cell tray, (F) Russia, 200 plug cell tray and (G) Russia, 288 plug cell tray.

Table 1. Growth characteristics by No. of cells/tray of *H. rhamnoides* L.

Clone origin	No. of cells/tray	Shoot length (cm)	Leaf length (cm)	Root length (cm)	Leaf width (cm)	No. of leaves	Stem diameter (mm)	Root diameter (mm)	Fresh weight (g)		Dry weight (g)	
									Shoot	Root	Shoot	Root
China	50	7.83±0.60^{a}	4.03±0.20^{a}	8.83±0.44^{b}	0.77±0.33	8.00±0.00^{a}	0.54±0.23	0.84±0.18^{b}	0.26±0.31^{c}	0.04±0.01^{b}	0.07±0.00	0.015±0.00^{a}
	128	5.07±0.08^{bcd}	3.63±0.33^{ab}	8.83±0.44^{b}	0.67±0.33	6.67±0.67^{ab}	0.45±0.36	0.88±0.05^{b}	0.21±0.01^{cd}	0.05±0.00^{b}	0.01±0.00	0.02±0.00^{a}
Russia	50	7.40±0.21^{a}	2.50±0.15^{ef}	11.97±0.92^{a}	0.73±0.33	8.00±0.00^{a}	0.27±0.52	0.11±0.06^{a}	1.06±0.07^{a}	0.87±0.15^{a}	0.62±0.18	0.024±0.012^{a}
	72	5.53±0.32^{bc}	3.13±0.12^{cd}	11.30±0.15^{a}	0.77±0.33	6.00±0.00^{bc}	0.17±0.01	0.08±0.04^{a}	0.92±0.02^{b}	0.70±0.06^{a}	0.43±0.00	0.019±0.010^{a}
	128	5.97±0.42^{b}	3.30±0.17^{bc}	6.50±0.25^{c}	0.6±0.33	5.33±0.67^{bc}	0.83±0.03	0.77±0.04^{c}	0.13±0.04^{c}	0.12±0.06^{b}	0.51±0.00	0.013±0.004^{a}
	200	4.43±0.26^{d}	2.80±0.10^{de}	6.43±0.44^{c}	0.73±0.33	4.67±0.67^{c}	0.96±0.22	1.04±0.03^{c}	0.17±0.01^{cd}	0.16±0.03^{b}	0.18±0.13	0.020±0.003^{a}
	288	4.77±0.16^{cd}	2.30±0.12^{f}	8.40±0.06^{b}	0.6±0.00	4.67±0.67^{c}	0.85±0.28	0.81±0.07^{b}	0.13±0.01^{c}	0.06±0.00^{b}	0.03±0.01	0.016±0.003^{a}
p-value		**	**	**	NS	**	NS	**	**	**	NS	**

**Represent significance at the 1% levels.

The values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$); NS: not significant.

nation rate was high in the order of 200, 128, 72, 288 plug cell tray. The growth of plantlets were similar to that of Chinese origin being vibrant generally in 50 plug cell trays with plant length 7.4 cm, root length 11.9 cm, No. of leaves 8 and different results were confirmed showing leaf length 3.3 cm, in 128 plug cell tray, and new shoot diameter 0.96 mm, root diameter 1.04 mm in 200 plug cell tray rate.

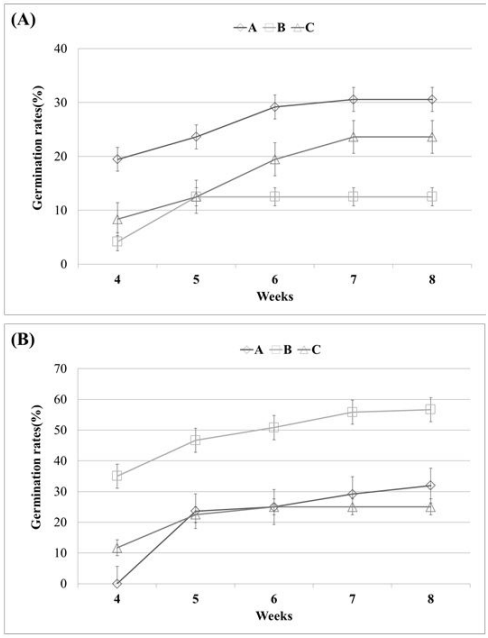

Fig. 3. Effect of soil condition of seed for germination rates (%) of different sources of *H. rhamnoides* L. (A) China and (B) Russia. A: TKS, B: Horticultural substrate and C: Sandy.

Effect of soil on seed germination rates and seedling growth

In *in vivo* test of seed germination according to soil composition, both of Chinese seeds and Russian seeds planted in soil-cement showed 20% germination rate, in case of Chinese origin soil, it was the highest 30% in TKS-2, in case of Russian origin it was about 60% in bed soil for horticulture, showing the highest germination rate (Fig. 3). It was confirmed that Chinese origin plantlet growth was

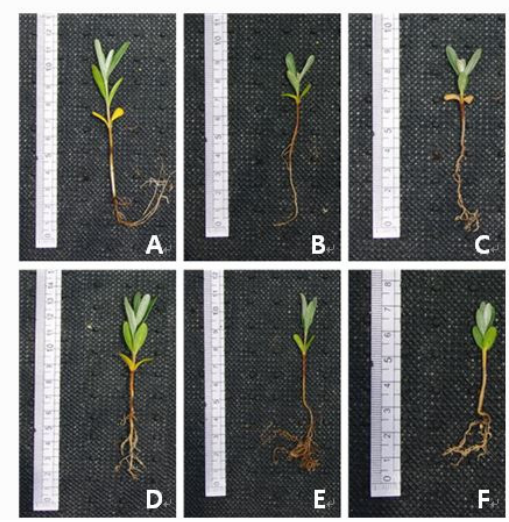

Fig. 4. Effect of soil condition of different sources of *H. rhamnoides* L. (A) China, TKS-2, (B) China, Horticultural substrate (C) China, Sandy soil, (D) Russia, TKS-2, (E) Russia, Horticultural substrate and (F) Russia, Sandy soil.

Table 2. Growth characteristics by soil for the growth of *H. rhamnoides* L.

Clone origin	Soil	Shoot length (cm)	Leaf length (cm)	Root length (cm)	Leaf width (cm)	No. of leaves	Stem diameter (mm)^{ns}	Root diameter (mm)^{ns}	Fresh weight (g)		Dry weight (g)	
									Shoot	Root	Shoot	Root
China	A	5.07±0.08^{ab}	3.63±0.03^{a}	8.50±1.00	0.67±0.03^{b}	6.67±0.67	1.02±0.08	0.88±0.03	0.24±0.04	0.05±0.01	0.07±0.01	0.02±0.00
	B	4.00±0.00^{cd}	2.97±0.03^{c}	5.83±0.33	0.73±0.03^{b}	4.67±0.67	1.01±0.03	0.93±0.11	0.17±0.02	0.05±0.01	0.05±0.01	0.03±0.01
	C	3.77±0.63^{cd}	2.43±0.08^{d}	5.50±1.60	0.43±0.08^{c}	5.33±0.67	0.93±0.03	0.75±0.03	0.09±0.01	0.03±0.01	0.02±0.00	0.01±0.00
Russia	A	5.97±0.41^{a}	3.30±0.17^{b}	6.50±0.25	0.77±0.03^{b}	5.33±0.67	0.83±0.03	0.77±0.04	0.13±0.04	0.12±0.06	0.05±0.01	0.01±0.00
	B	4.73±0.14^{bc}	2.73±0.03^{c}	7.37±2.07	2.30±0.11^{a}	4.67±0.67	0.85±0.01	0.83±0.11	0.14±0.01	0.05±0.12	0.03±0.01	0.01±0.00
	C	3.00±0.17^{d}	2.07±0.03^{e}	6.10±0.80	0.57±0.03^{bc}	4.67±0.67	0.83±0.06	0.83±0.08	0.07±0.00	0.23±0.01	0.02±0.01	0.01±0.00
p-value		**	**	NS	**	Ns	NS	NS	NS	NS	NS	NS

A: TKS-2, B: Horticultural substrate, C: Sandy soil.

**Represent significance at the 1% levels.

The values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$); NS: not significant.

more vibrant in TKS-2 in general than any other condition showing plant length (5.0 cm), leaf length (3.6 cm), root length (8.5 cm), No. of leaves (6.6), fresh weight (0.24 g/0.05 g; shoot/root), dry weight (0.07 g/0.02 g; shoot/root). It was confirmed that the growth in cement soil was the lowest compared to those in other treatments showing a new shoot (3.7 cm), root length (5.5 cm) (Table 2, Fig. 4).

Plantlets from Russian origin showed similar result to the Chinese origin with the result of new shoot (5.9 cm), leaf length (3.3 cm), No. of leaves (5.3) in TKS-2 and the result of bed soil for horticulture appeared with root length (7.37 cm), leaf width (2.3 cm), root diameter (0.8 mm) and it was confirmed that the growth of the plantlets in cement soil was relatively lower than those grown in other treatments showing new shoot (3.0 cm), leaf length (2.07 cm), fresh weight (0.07 g) (in shoot), 0.23 g (in root).

Effect of shading on seed germination and seedling growth

Propagation experiment of sea buckthorn according to shading treatment, Chinese origin seed germination rate in 70% shading was relatively high during the 4th week after planting compared to those of other treatments showing more than 10% germination rate, however, it was confirmed that from 5th week more than 20% germination rate started to appear in untreated shading (0%) and during the 8th week after planting the rate was 30% which was the highest germination rate compared to other treatments. In the 30% shading, less than 20% germination rate was shown from which it is judged that untreated shading is effective in seed germination for Chinese origin *in vivo* seed germination (Fig. 5). The result having measured growth status of plantlets showed that shoot growth was vibrant in 70% shading condition. Vibrant shoot growth included plant length (7.5 cm), leaf length (4.3 cm), leaf width (0.7 cm). Under the untreated shading, the root growth was relatively encouraged with root length of 8.5 cm and root diameter of 0.88 mm (Table 3, Fig. 6).

Russian origin showed about 50% germination rate in

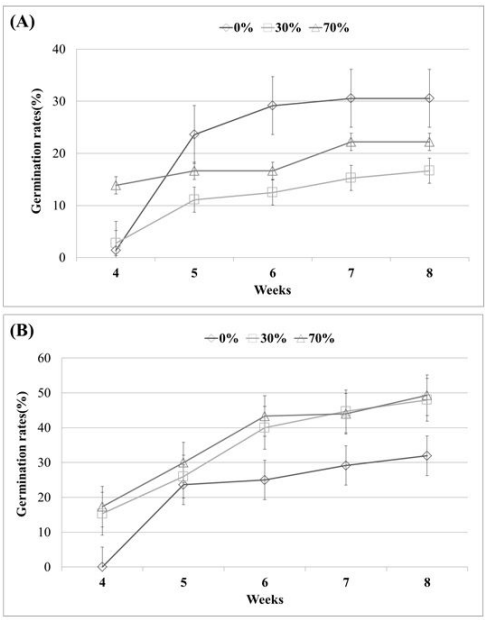

Fig. 5. Effect of shading rates for seed germination rates (%) of different sources of *H. rhamnoides* L. (A) China and (B) Russia. A: 0%, B: 30% and (C) 70%.

Table 3. Growth characteristics by shading rates of *H. rhamnoides* L.

Shading rates (%)		Shoot length (cm)	Leaf length (cm)	Root length (cm)	Leaf width (cm)	No. of leaves	Stem diameter (mm)	Root diameter (mm)	Fresh weight (g)		Dry weight (g)	
									Shoot	Root	Shoot	Root
China	0	5.07±0.08	3.63±0.03^{bc}	8.50±1.00^{ab}	0.67±0.03^{a}	6.67±0.67	1.02±0.08	0.88±0.03	0.242±0.047	0.051±0.010	0.068±0.012^{a}	0.016±0.004
	30	6.50±1.11	3.63±0.23^{bc}	5.30±0.47^{c}	0.53±0.03^{b}	4.67±1.33	0.91±0.03	0.49±0.06	0.135±0.020	0.030±0.002	0.019±0.001^{c}	0.006±0.001
	70	7.50±0.57	4.30±0.10^{a}	7.33±0.44^{bc}	0.70±0.05^{a}	6.00±0.00	0.75±0.07	0.61±0.15	0.189±0.017	0.038±0.003	0.035±0.002^{bc}	0.004±0.002
Russia	0	5.97±0.41	3.30±0.17^{c}	6.50±0.25^{bc}	0.77±0.03^{a}	5.33±0.67	0.83±0.03	0.77±0.04	0.132±0.040	0.116±0.065	0.051±0.010^{ab}	0.013±0.004
	30	9.67±0.88	4.10±0.25^{ab}	7.00±0.28^{bc}	0.77±0.03^{a}	7.33±0.67	0.74±0.04	0.77±0.01	0.244±0.035	0.052±0.008	0.045±0.006^{b}	0.009±0.002
	70	8.17±0.44	3.10±0.05^{c}	9.67±0.88^{a}	0.50±0.05^{b}	5.33±0.67	0.72±0.06	0.62±0.08	0.171±0.004	0.197±0.151	0.217±0.004^{c}	0.005±0.002
p-value			NS	**	*	**	NS	NS	NS	NS	NS	**	NS

*,**Represent significance at the 5%, 1% levels, respectively.
The values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$); NS: not significant.

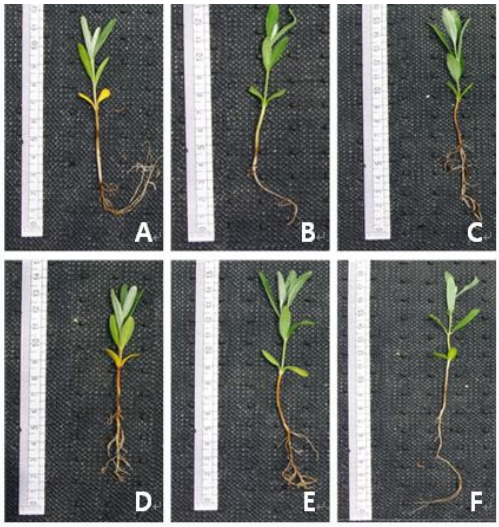

Fig. 6. Effect of shading rates of different sources of *H. rhamnoides* L. (A) China, 0% (B) China, 30%, (C) China, 70%, (D) Russia, 0% (E) Russia, 30% and (F) Russia, 70%.

30% and 70% shading compared to the untreated shading (Fig. 6). When Russian origin growth status was investigated, contrary to the Chinese origin, general growth was vibrant in 30% shading with plant length (9.6 cm), leaf length (4.1 cm), leaf width (0.7 cm), No. of leaves (7.3) and root length was most developed in 70% shading with 9.6 cm.

Discussion

Raising seedlings in plug cell trays enables crop production to be divided and formal work to be simplified, efficient and consistent. This also allows efficient time and labor force management reducing the quantity of seeds consumption and production of unique plant mass (Ito 1992; Pagani et al. 2013). Since its introduction to the Republic of Korea in the 1990s, the usage of plug raising of seedlings has been on the rise (Yeoung et al. 2005).

Size of plug cells and their influence on plants have been reported in various studies (Kim et al. 1999; Kim and Lee 1999; Shin et al. 2000) and this seems to be similar to results to the report that the bigger cell size is, the more growth is promoted because the bed soil per cell becomes relatively larger and nutrition in it is relatively rich (Yeoung et al. 2005).

The bed soil for horticulture currently sold domestically is mixed with organic material such as peat moss and coir and inorganic materials such as pearlite vermiculite, rock wool which have a dramatic influence on plant growth. Studies on bed soil physical and chemical characteristics together with raw material mixing rates have been actively carried out (Lee et al. 2006). According to Gabriëls et al. (1986), the element determining nutrition status in the rhizosphere environment appropriate for botanic growth are pH, EC, macroelement and microelement in bed soil hence, proper chemical component be important. It has been reported that TKS-2, a kind of bed soil containing organic material and inorganic nutrition with increased moisture containing capacity, is possible to treat for single and mixed purpose and encourages root development (Woo et al. 2001).

The result of this study is similar to the report of Duriyaprapan and Britten (1982) that the more shading degree is increased, the more remarkably plant length and leaf measurements increase. According to the study by Loach (1970), seedlings grown in the place where shading is high was treated control purported that relative growth rate of root decreases as photosynthesis rate is low due to lack of light. However, in this study, different results were observed in the Russia origin plantlet showing that the root in 70% shadingwas developed more than any other grown in other treatments.

In conclusion, the *H. rahmnoides* L. Chinese origin seed germination rates were best in 128 plug cell trays with no-shading. Seedling growth was best in 50 plug cell trays under 70% shading. TKS-2 soil was considered to be more desirable for both seed germination and growth of seedlings. The Russian origin seed germination and seedling growth were best in 50 plug cell trays. Seed germination was better in horticulture substrate under 30 and 70% shading whereas seedling growth was vibrant under 30% shading.

Acknowledgements

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