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Effect of Plant Growth Regulators in *In Vitro* Culture of *Hippophae rhamnoides*

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Abstract

This study was carried out to establish *in vitro* propagation system influenced by plant growth regulators through organogenesis with three different seed sources (China, Mongolia and Russia) for conservation of genetic resources in Northeast Asia. The experiment compared two different carbon sources (commercial sugar, sucrose), which showed no significant differences in germination rate. Induced adventitious buds from leaf segments were found to be highly effective when supplemented with 1.0 mg/L BA, 1.0 mg/L Kinetin, and 5.0 mg/L IAA, in the case of Chinese origin 96.8%, Russian origin R-1: 95.6%, R-2: 85.6%, and Mongolian origin M-2: 77.8%. It was effective in BA and Kinetin with supplemented with IAA, respectively. Shooting development was also efficient in Woody Plant Media (WPM) supplemented with 1.0 mg/L BA, 1.0 mg/L Kinetin and 5.0 mg/L IAA.

Key Words: adventitious bud, *Hippophae rhamnoides*, *in vitro* culture, plant growth regulators, seabuckthorn

Introduction

Seabuckrthorn (*Hippophae rhamnoides* L.) is a deciduous broad-leaved shrub belonging to the Elaegnaceae family. It is distributed in Europe, China, Mongolia, and Russia, and there are 6 species and 12 sub-species worldwide (Li and Schroeder 1996; Heinäaho et al. 2006). Seabuckthorn, which is native to Northeast Asia including Mongolia, China, and Russia, are mainly distributed in rivers, marginal forests, and slopes. It is widely distributed in areas where desertification occurs in China and Mongolia. Particularly, in Mongolia and Inner-Mongolia of China, excessive grazing is affecting the growth of seabuckthorn and its populations are decreasing due to forest fires (Ochirbat and Dejidkhuu 1986).

Seed oil of seabuckthorn is rich in carotene, flavonoid, vitamin K and vitamin E, which promotes blood coagulation through a catalytic action in prothrombin formation (Rongsen 1992). Seabuckthorn fruit have glucose, fructose and xylose, and organic acid of 3.9% (Ma and Cui 1989). In addition, seabuckthorn includes protein, total 18 amino-acid in fruit, and free amino acids (Mironov 1989). And seabuckthorn used as a treatment for burn, stomatitis, ulcers and other types of product such as liquid, powder, film in China, Russia, Mongoila (Letchamo et al. 2002). Shuunguang and Chaode (2001) reported study on the therapeutic potential of seabuckthron for human immunodeficiency virus (HIV) and Larmo et al. (2010) published the seabuckthorn oil helps cure dry eye symptoms. Therefore, the demand of seabuckthorn expected to in-

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crease potential medicinal value.

Researchs on *in vitro* tissue culture of seabuckthorn have mainly been conducted on propagations, concentrated on the studies on *in vitro* shoot multiplication and root symbiosis with *Rhizobacterium* (Montpetit and Lalonde 1988). Yang et al. (2004) reported that research on preservation of genetic resources for mass production and development of new varieties of seabuckthorn need to be conducted. Even though several clones were produced in China (Lummerding 2001; Liu et al. 2007), Europe (Sriskandarajah and Lundquist 2009) and India (Gupta and Singh 2003) have been studied on clonal reproduction, the useful data is not enough to build up propagation in *in vitro* condition. Therefore, this study was conducted to establish a mass propagation system for the preservation and protection of genetic resources of seabuckthorn from different countries with different seed sources of seabuckthorn that grow naturally in Northeast Asia.

Materials and Methods

Experiment materials

The seeds for this study were obtained from Mongolian and Russian seeds from the Institute of Geo-ecology, Mongolia and Chinese seeds from the Institute of Combating Desertification, Inner-Mongolia, China. Seeds length and width were measured. All seeds were stored in the seed storage facility (4±2°C) at the Environmental Biotechnology Laboratory, Dongguk University.

Seed germination by the different carbon sources

To investigate seed germination according to the carbon sources, of sucrose and commercially available 3% general sugar (white sugar, CJ CheilJedang, Korea) were added to white medium (White 1939). In order to introduce the seeds into the *in vitro* condition, the seeds were washed with water for 30 minutes, immersed in 70% ethanol (EtOH) for 30 seconds, and then immersed twice for 15 minutes in 50% NaOCl solution. This was followed by secondary disinfection using methanol surface heating treatment, and then plated on the medium. The pH of all the media used in the experiments was adjusted to pH 5.7 before sterilization, added 0.3% Bacto-agar with 0.15% gelrite, and autoclaved at 121°C for 20 minutes. All culture conditions were maintained at 16:8 (day:night) at a temperature of 23±2°C and an illuminance of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The seed germination rate in *in vitro* was determined based on the germination criteria of the seed having shoot primordia with an elongation of 2 mm or more, and the data was measured 2 weeks after the placement.

Adventitious bud induction by the treatment of plant growth regulators

Seabuckthorn of cotyledons and leaf segments were used for induction of adventitious buds under *in vitro* condition. The media of WPM (Woody Plant Medium) were mixed with 2.5% glucose and 0.5% sucrose and supplemented with 0, 1.0, 5.0 mg/L BA (Benzyladenin), 0, 1.0, 5.0 mg/L Kin (Kinetin), 5.0 mg/L IAA (Indole-3-acetic acid), respectively, to compare the effects of plant growth regulators. The sub-cultures were repeated every 3 weeks during the culture.

Root induction

Root induction was taken with sterilized soil with 1/2 WPM (pH 5.7), dispensed into a glass bottle. Control, 100 ppm and 1,000 ppm of IBA (Indole-3-butyric acid) and NAA (1-Naphthaleneacetic acid) are immersed for 3 to 5 seconds, and then placed in a sterilized culture medium to induce roots.

Plantlet with tiny roots were transplanted using mixed soil in outside condition and covered with a transparent acrylic plate at the beginning of the transplant to maintain sufficient humidity. Ventilation treatments were performed, and after 3 weeks, the acrylic plates were removed to investigate whether it survived.

Results and Discussion

Morphology of seabuckthorn seeds

The characteristics of seabuckthorn seeds were investigated using seeds from seabuckthorn according to production area; The average width of seeds was about 2 cm regardless of its origin (Table 1). The Chinese seed (C-1) was 5.73±0.45 cm long, and the Mongolian seed (M-1) was 5.30±0.53 cm. Mongolian (M-2) seeds were the shortest at 2.47±0.57 cm. The number of seeds per 10 g was 1,252 in M-2 seeds with the highest number of seeds,

Table 1. Comparison of width, length and number of *H. rhamnoides* seed

Seed sources	Width (cm)	Length (cm)	No. of seed/10 g
M-1	2.30±0.47[a]	5.30±0.53[b]	779
M-2	2.03±0.18[b]	2.47±0.57[d]	1,252
C-1	2.23±0.43[ab]	5.73±0.45[a]	566
R-1	2.27±0.45[a]	4.97±0.76[c]	661
R-2	2.20±0.41[ab]	4.93±0.83[c]	834

Means±standard deviation. Mean values followed by the same letter do not differ significantly according to Duncan's multiple range test at p=0.05. M-1, M-2 and M-3: origin of Mongolia introduced from Institute of Geo-ecology, Mongolia. C-1: origin of China introduced from Institute of Combating Desertification, Inner-Mongolia, China. R-1 and R-2: origin of Russia introduced from Institute of Geo-ecology, Mongolia. All seed sources were introduced at the same year from the provenance countries.

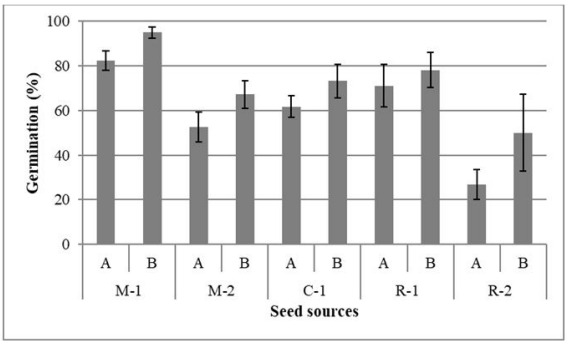

Fig. 1. Effects of carbon sources for seed germination with different seed sources on Woody Plant Media with *H. rhamnoides*. A: 3% Sucrose, B: 3% Commercial sugar.

and the smallest seed size. There were 834 seeds from Russia (R-2) and 566 seeds from China (C-1), with the lowest number of seeds per 10 g (Table 1).

Seed germination by the different carbon sources

Most of the seed germination initiated after 5 days on the media and completed after 2 weeks regardless of the seed provenances and carbon source type, and germination was completed 2 weeks after placement in the media. M-1 seeds with 3% commercial sugar showed the highest germination rate (95%), and germination proceeded in the sequential order of R-1, C-1, M-2, and R-2. However, the contaminations during the culture period appeared in several seed sources and the rate of R-2 was the highest at 90% whereas that of M-1 seeds was the lowest, despite of the same pre-disinfection treatment (Fig. 1). Pierik (1998) reported that commercial sugar contains 99.94% sucrose (sugar), 0.02% moisture, raffinose such as 0.04% fructose, glucose, and minerals, and contains toxic substances.

Therefore, it is available to use it sold in market as a commercial sugar when cultivating plant tissue culture. Liu et al. (2007) reported that commercial sugar was used in a study on the *in vitro* culture of Chinese seabuckthorn, and that the efficiency of plant regeneration was similar to be used by sucrose for tissue culture. As similar results with our studies sucrose and l commercial sugar did not significantly affect seed germination. It means that commercial sugar much more than cheaper than sucrose would be recommended for *in vitro* seed germination of seabuckthorn.

When we compared seeds harvested in the same year, there was a difference in germination rate according to the seed sources. It seems that the dormancy and physiological causes of seeds affect seed germination. Seedling production in *in vitro* condition can also be achieved by breaking the dormancy of seeds (Hilhorst and Karssen 1992; Debeaujon et al. 2000). Relevant studies have been conducted that chemical treatment such as GA_3 and sulphuric acid, ethanol immersion, mechanical scarification and radiation treatments have been applied for promoting seed germination by breaking its dormancy (Hilhorst and Karssen 1992; Kelly et al. 1992; Koornneef and Karssen 1994; Debeujon et al. 2000; Doo et al. 2000). In this study, it is considered that disinfection using ethanol immersion treatment and methanol surface heating treatment during *in vitro* culture were efficient for the disinfection of seeds as well as for breaking seed dormancy. On the other hand, it was found that the medium around some seeds turned brown during seed germination. According to a study by

Sriskandarajah and Lundquist (2009) have been reported that browning of the medium was shown due to the release of phenolic substances from the seabuckthorn seeds under insufficient light or abnormal environmental conditions.

Adventitious bud induction by the treatment plant growth regulators

As a result of organogenesis of seabuckthorn, the induction rate of adventitious buds was higher in leaf segment than in cotyledons. The induction rate of adventitious bud being similar to be embryos was relatively higher in Mongolian seed source compared to other origins, and shoot primordia were induced in all treatments using *in vitro* cotyledon and leaf segments. In the treatment with 1.0 mg/L BA, 1.0 mg/L Kin, and 5.0 mg/L IAA, the rate of adventitious bud induction was highest at 96.8% in leaf segment of C-1, and R-1 (95.6%). Adventitious buds were induced in the order of R-2 (85.6%), M-1 (81.1%), and M-2 (77.8%) (Table 2). By the treatments with 1.0 mg/L BA and 5.0 mg/L IAA, shooting rate of M-1 was 93.1%, which was relatively higher compared to C-1 (90.0%), R-1 (75.5%), R-2 (60.1%) and M-2 (25.9%). On the other hand, the induction rate was lower in the treatment with 1.0 mg/L Kin and 5.0 mg/L IAA compared to BA, but more than 70% in M-1 (73.4%) and C-1 (77.8%). The combination of BA and IAA also induced 63.9% and 40.3% of ad-

Fig. 2. Adventitious bud induction from leaf segments on WPM supplemented with 1.0 mg/L BA, 1.0 mg/L Kin and 5.0 mg/L IAA (A) and (B) Proliferation of adventitious bud, (C) and (D) Elongation of adventive shoot bud, (E) Adventive shooting and root induction, (F) Abnormally developed shoot.

Table 2. Effects of Plant Growth Regulators (PGRs) for organogenesis from cotyledon and leaf segments induced from different seed sources of *H. rhamnoides* in *in vitro* condition on WPM media

Sources	PGRs (mg/L)			Organogenesis (%)		Germination rates (%)	
	BA	Kin	IAA	Cotyledon	Leaf	Cotyledon	Leaf
M-1	1.0		5.0	63.9±21.9^{a}	93.1±4.2^{a}	22.2±10.0^{b}	56.7±7.0^{abcd}
		1.0	5.0	36.1±19.9^{b}	73.4±12.5^{abcd}	22.6±6.0^{b}	41.1±4.5^{bcde}
	1.0	1.0	5.0	69.1±8.4^{a}	81.1±10.2^{ab}	11.11±7.5^{b}	76.7±9.0^{a}
M-2	1.0		5.0	40.3±10.2^{b}	25.9±11.1^{e}	50.4±9.8^{a}	43.9±8.5^{bcde}
		1.0	5.0	38.8±7.5^{b}	22.2±3.7^{e}	15.7±7.0^{bc}	33.5±5.8^{cdef}
	1.0	1.0	5.0	45.5±3.2^{b}	77.8±11.1^{e}	20.0±10.1^{b}	66.9±10.5^{abc}
C-1	1.0		5.0	-	90.0±4.8^{a}	-	14.8±3.7^{ef}
		1.0	5.0	63.0±14.8^{a}	77.8±5.2^{abc}	10.3±4.7^{bc}	-
	1.0	1.0	5.0	-	96.8±3.2^{a}	-	33.3±11.1^{cdef}
R-1	1.0		5.0	-	75.5±15.1^{abcd}	-	30.1±10.9^{def}
		1.0	5.0	38.9±5.5^{b}	66.7±33.3^{bcd}	17.5±2.1^{bc}	40.1±12.5^{bcde}
	1.0	1.0	5.0	-	95.6±4.4^{a}	-	33.3±10.5^{cdef}
R-2	1.0		5.0	-	60.1±15.1^{cd}	-	20.5±11.4^{ef}
		1.0	5.0	-	55.7±3.3^{d}	-	45.3±13.9^{abcde}
	1.0	1.0	5.0	-	85.6±13.4^{ab}	-	70.8±17.9^{ab}

Values are means±standard deviation.
Means values followed by the same letter do not differ significantly according to Duncan's multiple range test at p=0.05.

ventitious embryo only in Mongolian sources (M-1, M-2) (Table 2; Fig. 2). By the treatment of 1.0 mg/L BA, 1.0 mg/L Kin and 5.0 mg/L IAA, the induction rate of adventitious buds was 96% in the leaves of R-1, which was relatively high compared to other treatments.

M-2 also showed an induction rate (77%) in the treatment with 1.0 mg/L BA, 1.0 mg/L Kin, and 5.0 mg/L IAA. The adventitious bud induction appeared 93.1% in M-1 with the 1.0 mg/L BA and 5.0 mg/L IAA. C-1 cotyledons were shown 63.0% that was relatively high value compared to other sources, whereas Mongolian and Russian (R-1) showed an induction rate of about 36-38%.

It showed shooting from adventitious bud M-1 (76.7%), R-2 (70.8%), M-2 (66.9%), C-1, R-1 (33.3%) in the treatment of 1.0 mg/L BA, 1.0 mg/L Kin, and 5.0 mg/L IAA. It could be relatively higher shooting in M-1 (56.7%) under the treatment of 1.0 mg/L BA and 5.0 mg/L IAA than from China and Russia sources (Table 2). The results were similar to a study on *in vitro* mass propagation and shoot induction was effective in 1.0 mg/L BA treatment, and callus induction was effective in Kin 1.0 mg/L treatment (Montpetit and Lalonde 1988).

Sriskandarajah and Lundquist (2009) reported when TDZ (Thidiazuron) treatment in cotyledon increased shooting with the adventitious bud induction rate of approximately 60%, in the leaf segments and promoted shoot formation by the treatment of TDZ and BA mixture. The induction rate of adventitious buds was lower in the TDZ treatment and the browning of the sample proceeded as well as the organ induction not developed in the BA and Kinetin treatments. It is reported that nutrients and plant growth regulators are essential for *in vitro* culture of seabuckthorn (Gupta and Singh 2003), and that the efficiency of *in vitro* cultivation of seabuckthorn is reduced when BA and Kinetin are treated alone (Montpetit and Lalonde 1988; Knyanzev et al. 2003; Yang et al. 2004). In this study, it assured that treatment with BA, Kinetin, and IAA was more effective than other treatments.

In the process of induction of shoots, new tiny shoots were turned to be dead due to browning of culture media. The phenomenon of death due to browning of the stem being cultured or chlorotic phenomenon is caused shoot tip necrosis (STN) in *in vitro* condition. One relevant result reported the absorption of Ca^{2+}, which is involved in the synthesis of plant hormones responsible for cell wall formation and transport of inorganic salts, becomes inefficient (Martin et al. 2007).

McCown and Sellmer (1987) reported that roots do did not form when seabuckthorn shoot induction was formed only in stem cultivation in the treatment of cytokinin.

Root induction

As a result of root induction from the induced shoots, only 2 roots were produced in 100 ppm NAA treatment group in Mongolian source (M-1) and appeared to be rapidly dead 4 days after placement. It was reported that roots were induced when no hormone was added to the Murashige and Skoog (MS) medium for early rooting of seabuckthorn by Montpetit and Lalonde (1988) and Vantu (2007). However, Proebsting (1984) reported that NAA immersion treatment was effective in inducing roots. In this study, approximately 90% of the shoot induction rate was shown even though the rooting rate was significantly low. In a preliminary experiment, Auxin-based plant growth regulator such as IAA, NAA, and IBA, which are widely used for rooting, were cultured in solid medium containing 0, 1.0, 3.0, 5.0 mg/L. However, the roots were not induced and died within one week of the culture. In addition, in *in vitro* propagation experiment of Chinese seabuckthorn, the plants were abnormal deformations observed after induction of somatic embryos (Liu et al. 2007), and some of the tissues died during subculture processes (Lummerding 2001). Kalia et al. (2011) pointed out difficulty in *in vitro* culture of seabuckthorn, the problems of *in vitro* culture of seabuckthorn such as low growth rate, occurrence of over-hydration, browning, death in the subculture process, and low rooting rate.

In conclusion, the present studies concentrated on shoot formation from leaf segments and cotyledons of seabuckthorn. The seeds from China, Mongolian and Russia showed significantly different germination rate to each other. The adventitious bud formation was shown with the treatments of plant growth regulators. The adventitious bud formation also was clearly shown through the stereo-microscope and the sectioning of shoot forming leaf segments. Our results suggest that the adventitious bud formation developed from leaf segments and cotyledons might be possible for stable seedling production for the establishment of

plantation. In future study, the acclimation might be necessary to produce fully grown seedling under greenhouse condition before transplanting to the nursery.

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References

Debeaujon I, Léon-Kloosterziel KM, Koornneef M. 2000. Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. Plant Physiol 122: 403-414.

Doo HS, Li HL, Kwon TH, Yang MS. 2000. Development of Aspceptic Seedling by *In Vitro* Germination in Lacquer Tree Seed. Korean J Plant Res 13: 48-53.

Gupta RK, Singh V. 2003. Studies on Micropropagation in Seabuckthorn (*Hippophae rhamnoides* L.). A Multipurpose Wonder Plant. Indus Publishing Company, New Delhi, pp 334-338.

Heinäaho M, Pusenius J, Julkunen-Tiitto R. 2006. Effects of different organic farming methods on the concentration of phenolic compounds in sea buckthorn leaves. J Agric Food Chem 54: 7678-7685.

Hilhorst HWM, Karssen CM. 1992. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. Plant Growth Regul 11: 225-238.

Kalia RK, Singh R, Rai MK, Mishra GP, Singh SR, Dhawan AK. 2011. Biotechnological interventions in sea buckthorn (*Hippophae rhamnoides* L.): current status and future prospects. Trees 25: 559-575.

Kelly KM, Van Staden J, Bell WE. 1992. Seed coat structure and dormancy. Plant Growth Regul 11: 201-209.

Knyazev AV, Chemeris AV, Vakhitov VA. 2003. Morphogenesis of *Hippophae rhamnoides* L. *in vitro*. Rastit Resur 39: 107-115.

Koornneef M, Karssen CM. 1994. Seed Dormancy and Germination. *In: Arabidopsis* (Meyerowitz EM, Somerville CR, eds). Cold Spring Harbor Laboratory Press, Plainview, pp 313-334.

Larmo PS, Järvinen RL, Setälä NL, Yang B, Viitanen MH, Engblom JR, Tahvonen RL, Kallio HP. 2010. Oral sea buckthorn oil attenuates tear film osmolarity and symptoms in individuals with dry eye. J Nutr 140: 1462-1468.

Letchamo W, Klevakin R, Lobatcheva II. 2002. Heavy Metal Accumulation in Sea Buckthorn Cultivars in Siberia. In: Trends in New Crops and New Uses (Janick J, Whipkey A, eds). ASHS Press, Alexandria, pp 399-401.

Li TSC, Schroeder WR. 1996. Sea Buckthorn (*Hippophae rhamnoides* L.): A Multipurpose Plant. Horttechnology 6: 370-380.

Liu CQ, Xia XL, Yin WL, Zhou JH, Tang HR. 2007. Direct somatic embryogenesis from leaves, cotyledons and hypocotyls of *Hippophae rhamnoides*. Biol Plant 51: 635-640.

Lummerding P. 2001. Micropropagation protocol development for seabuckthorn (*Hipphophae rhamnoides*) selections for commercial orchard production. Prairie Plant Systems. pp. 1-2.

Ma Z, Cui Y. 1989. Studies on the fruit character and biochemical composition of some forms with the Chinese sea-buckthorn (*Hippophae rhamnoides* ssp. *sinensis*) in Shanxi, China. In: Proceedings of International Symposium of Sea Buckthorn (*H. rhamnoides* L.); Xian, China; October 19-23, 1989. pp. 106-112.

Martin KP, Zhang CL, Slater A, Madassery J. 2007. Control of shoot necrosis and plant death during micro-propagation of banana and plantains (*Musa* spp.). Plant Cell Tiss Organ Cult 88: 51-59.

McCown BH, Sellmer JC. 1987. General Media and Vessels Suitable for Woody Plant Culture. *In*: Cell and Tissue Culture in Forestry: General Principles and Biotechnology (Bonga JM, Don D, eds). Springer, Dordrecht, pp 4-16.

Mironov VA. 1989. Chemical composition of *Hippophae rhamnoides* of different populations of the USSR. *In*: Proceedings of International Symposium of Sea Buckthorn (*H. rhamnoides* L.); Xian, China; October 19-23, 1989. pp. 67-69.

Montpetit D, Lalonde M. 1988. *In vitro* propagation and subsequent nodulation of the actinorhizal *Hippophae rhamnoides* L. Plant Cell Tiss Organ Cult 15: 189-199.

Ochirbat G, Dejidkhuu KH. 1986. Sum forms of cultivated seabuckthorn on the western part of Baga-khentii. "Seabuckthorn-86" (In Mongolian). pp. 26-29. Thesis of papers of theory and practice conference. Ulaanbaattar, Mongolia.

Pierik RLM. 1998. *In Vitro* Culture of Higher Plants. 4th ed. Dordrecht, Kluwer Academic, pp 31-82.

Proebsting WM. 1984. Rooting of douglas-fir stem cuttings: relative activity of IBA and NAA. HortScience 19: 854-856.

Rongsen L. 1992. Seabuckthorn: A Multipurpose Plant Species for Fragile Mountains. International Centre for Integrated Mountain Development, Kathmandu, 62 pp.

Shuunguang L, Chaode M. 2001. Direction, focus and contents of Seabuckthorn research and development in China- facing the new century. In: International Workshop on Seabuckthorn; New Delhi, India; February 18-21, 2001. pp. 18-21.

Sriskandarajah S, Lundquist PO. 2009. High frequency shoot organogenesis and somatic embryogenesis in juvenile and adult tissues of seabuckthorn (*Hippophae rhamnoides* L.). Plant Cell Tiss Organ Cult 99: 259.

Vantu S. 2007. Clonal micropropagation of *Hippophae rhamnoides* ssp. carpathica. Planta Med 73: 626.

White PR. 1939. Potentially Unlimited Growth of Excised Plant Callus in an Artificial Nutrient. Am J Bot 26: 59-64.

Yang LP, Zhang FL, Zhao XM. 2004. Rapid propagation of seabuckthorn (*Hippophae rhamnoides* L.). Global Seabuckthorn Res Dev 2: 12-16.