African Journal of Biotechnology Vol. 11(90), pp. 15712-15718, 8 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3876
ISSN 1684–5315

Full Length Research Paper

Effect of auxin treatments on male and female cuttings of *Hippophae salicifolia*

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Accepted 20 June, 2012

Hippophae salicifolia **(Seabuckthorn) is one of the potential multipurpose underutilised plant species having huge multipurpose benefits including economic and ecological. Effect of different concentrated doses of auxins, on rooting percentage, number of root per rooted cuttings and root length of male and female stem cuttings was examined under open field condition. Results show that the cuttings pretreated with 50 mg L^{-1} indole-3-butyric acid (IBA) had best rooting traits (respectively for male and female rooting percentage was 50.00 and 76.67%, root number was 2.3 and 3.0 and root length per rooted cuttings was 2.5 and 3.5 cm). Therefore, cuttings pre-treated with 50 mg L^{-1} IBA is recommended for vegetative propagation through cuttings in *H. salicifolia.* Moreover, this study provides a significant lead towards the development of a simple cost-effective propagation technique for large scale cultivation and future domestication of the elite genotype for better nutritional security along with socio-economic upliftment and sustainable rural development in Indian Himalayan Region.**

Key words: Seabuckthorn, vegetative propagation, dioecious plant.

INTRODUCTION

Hippophae salicifolia (Seabuckthorn) is at the top of multipurpose plant species in several countries and gradually replacing a number of plants due to its huge multipurpose benefits including high economic return from market.

Seabuckthorn (*H. salicifolia*) belonging to Elaegnaceae, is a thorny, dioecious small tree growing wild in the cold and dry regions of Indian Himalaya Region mainly in Uttarakhand, Jammu and Kashmir, Himachal Pradesh and Sikkim. The ripe fruit of Seabuckthorn is a natural bank of vitamins (A, B, C, and K and E) with antioxidant, anti-cancer, anti-AIDS and anti stress properties (Koelz, 1979; Fuheng, 1991; Jain, 1991; Rongsen, 1992; Mingyu, 1994; Xing et al., 2002; Zeb, 2004; Dhyani et al., 2007).

Consequently, more than 150 processing factories viz., nutraceutical and pharmaceutical companies are engaged in the utilization of this important natural resource by producing more than 200 different kind of products mostly as life saving drugs, health tonics, food, and cosmetics (Chen, 1988; Rongsen, 1992; Thomas, 2002; Ahmad and Kamal, 2002; Raffo, 2004; Venugo-palan et al., 2005; Lebeda, 2005; Sabir et al., 2005; Tiitinen, 2006; Dhyani et al., 2007; Gayle, 2008).

Recently, the plant species has attracted an extraordinary attention of researchers, scientists and environmentalists not only from Asia and Europe but also from North America because of its economical and ecological importance. However, in Indian Himalayan Region (IHR), no attention has been given towards the scientific conservation, propagation and utilization of this valuable plant.

In IHR, the natural resources of Seabuckthorn are degraded and vanished through increasing anthropogenic activities as the large scale clearing and burning of forests, over harvesting of plants, conversion of wild lands to agricultural, road and dam constructions etc., caused the plant to become over exploited, and to be classified as near to threatened category by IUCN (Dhyani, 2007). Hence, the conservation of this plant species is required to pay necessary attention in terms of mass multiplication and cultivation.

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Propagation of the species by seed is time consuming technique which also cannot maintain the fine biological characteristics and economic properties that are genetically identical to the selected mother plants. In order to meet the demand of development of adequate *H. salicifolia* (Seabuckthorn) resources with a regulated ratio of male and female plants for high amount of fruit yield, propagation from cutting needs to be preferred (Dhyani, 2007).

Considering all these factors the present study was investigated to determine the best vegetative propagation protocol for male and female plants of the target species with reference to rooting response and other parameters under different auxin treatments.

MATERIALS AND METHODS

Experimental site and climate

Experiment on vegetative propagation of *H. salicifolia* was carried out under natural environmental conditions at an altitude of 2600 m amsl in village Tolma, located in Niti valley of Nanda Devi Biosphere Reserve (NDBR) at Central Himalaya (28° 43' 45" to 30° 20' 12" N and 78° 44' 30' to 80° 18' 45" E), in Uttarakhand state of India. The cuttings were collected from different pockets of Gangotri Valley located at district Uttarkashi in Uttarakhand, India. The genotypic selection of Seabuckthorn cuttings from different pockets in Gangotri valley was based on the earlier research on best nutritional constituents of the fruit berries (Dhyani et al., 2007). Mean maximum and minimum aerial temperatures recorded during the study period were 28.05 and –0.93°C, respectively.

Sex differentiation and collection of stem cuttings

The sex of a Seabuckthorn tree cannot be identified until first flower buds or fruit berries appear. Therefore, to distinguish between male and female sex the trees were randomly marked with two different colours (red for male and green for female) during the fruiting period (as only female plant yield fruits) in October 2004. Male and female cuttings were collected in the first week of March, 2005 from 20 healthy mother plants. Stem cuttings of 15 to 20 cm length and 0.8 to 2.0 cm diameter, cleared for all leaves were collected and immediately kept in plastic film bags to prevent from wind drying and withering.

Auxin treatments and planting

Before going for experimental treatments all the cuttings were properly washed with running water for dust clearing. One set of male and female cuttings were kept as control (Cont.) and dipped in distilled water. Auxins were applied in 1.5% v/v aqueous ethanol solution. The basal part of cuttings was placed at 2 to 3 cm depth in the different concentrated doses (25, 50, 100, 300, 500 and 1000 mg L^{-1}) of indole 3-acetic acid (IAA), indole 3-butyric acid (IBA) and naphthalene acetic acid (NAA) at room temperature. A total of nineteen treatments were obtained. Each treatment has ten replicate of cuttings. All the cuttings were planted in nursery polythene bags filled with the soil substratum.

Data recording and analysis

Observations and data recording for different growth parameters of root were made fortnightly. The final rooting was recorded after 90 days. Moreover, the male and female cuttings were again uprooted after 6 months of planting to examine the root system. Weeding and irrigation were done as per the requirements. One way analysis of variance (ANOVA) and Dunnet test was applied for comparison of control cuttings with treated cuttings. The MiniTab 15.0 statistical programme was applied to process all the data in the study.

RESULTS

Effect of auxin treatments

After 90 days, the result show that the cuttings pretreated with auxins were significantly greater in the percentage of rooting, number of root per shoot and s`~average root length of per rooted cuttings than the control cuttings. The corresponding values of control cuttings were 26.67 $\pm$ 3.3%, 2.2 $\pm$ 0.2 and 2.3 $\pm$ 0.3 cm for male and 40.00 $\pm$ 5.8%, 3.3 $\pm$ 0.4 and 3.7 $\pm$ 0.3 cm for female plant cuttings (Figures 1, 2 and 3).

Different doses of auxin pre-treatments induced different rooting responses. The higher rooting percentage 56.67 $\pm$ 3.3 for male and 76.67$\pm$3.3 female cuttings were found respectively under 50 mg L^{-1} of IAA and IBA (Figure 1). On the other hand the lower rooting percentage were observed 3.33$\pm$3.3 under 1000 mg L^{-1} of IBA and NAA for male and female cuttings, respectively (Figure 1). With regard to the effect of different doses of auxins on number of root per rooted cuttings were found maximum 3.3$\pm$0.3 for male and 4.3$\pm$0.9 for female under 25 mg L^{-1} of NAA (Figure 2).

Results of average longest adventitious root were 3.1 $\pm$ 0.3 cm for male and 4.2 $\pm$ 0.4 cm for female cuttings under the lower concentrated dose of IAA 25 mg L^{-1} (Figure 3).

In general, the overall rooting traits were observed (rooting parentage are 50.00 and 76.67%, root number are 2.3 and 3.0, and root length per rooted cuttings are 2.5 and 3.5 cm) to have occurred under IBA 50 mg L^{-1}.

Effect of sex of donor plant

Sex of donor plant significantly ($P<0.05$) influenced rooting of cuttings in all pre-treated auxin treatments (Table 1). Rooting responses was generally higher in cuttings obtained from female tree than from male tree.

Interactive effects on rooting

Interactive effect of sex and different doses ofauxin treatments was significant at $P<0.05$ (Table 1). The interaction of female tree cuttings and IBA 50 mg L^{-1} resulted in higher rooting percentage followed by female x IAA 50 mg L^{-1} and female x NAA 50 mg L^{-1} (Figure 1). The combination of female x NAA 25 mg L^{-1} resulted in maximum number of root per rooted cuttings followed by

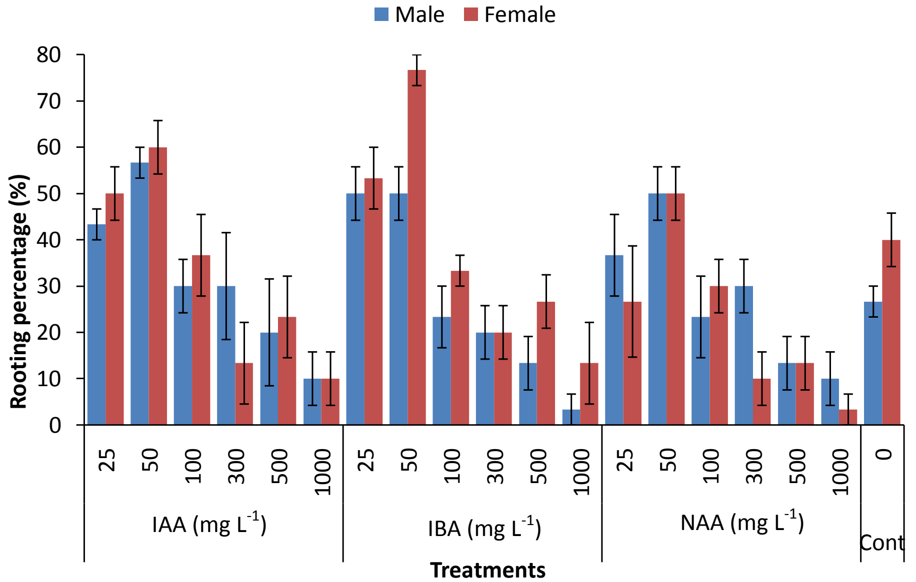

Figure 1. Effect of different auxin treatments against control on rooting percentage of male and female plant cuttings after 90 days.

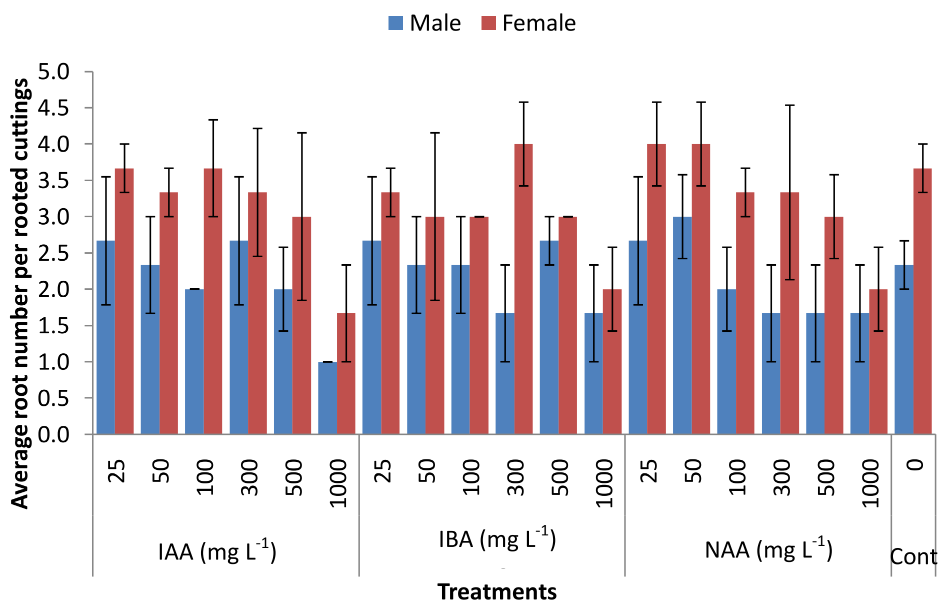

Figure 2. Effect of different auxin treatments against control on number of root per rooted cutting of male and female plant after 90 days.

female x 25 mg L^{-1} of IAA and IBA (Figure 2). Similarly the longest adventitious root was found under the interactive effect of female x IAA 50 mg L^{-1} followed by 50 mg L^{-1} of IBA and NAA (Figure 3). The overall response of female cuttings observed was significantly superior to male cuttings at all levels of IAA, IBA and NAA treatments.

DISCUSSION

In the present study, natural open field condition was selected for the propagation experiments for undertaking in-depth vegetative propagation experiments which proved the successful *ex-situ* propagation of this species (Figure 4).

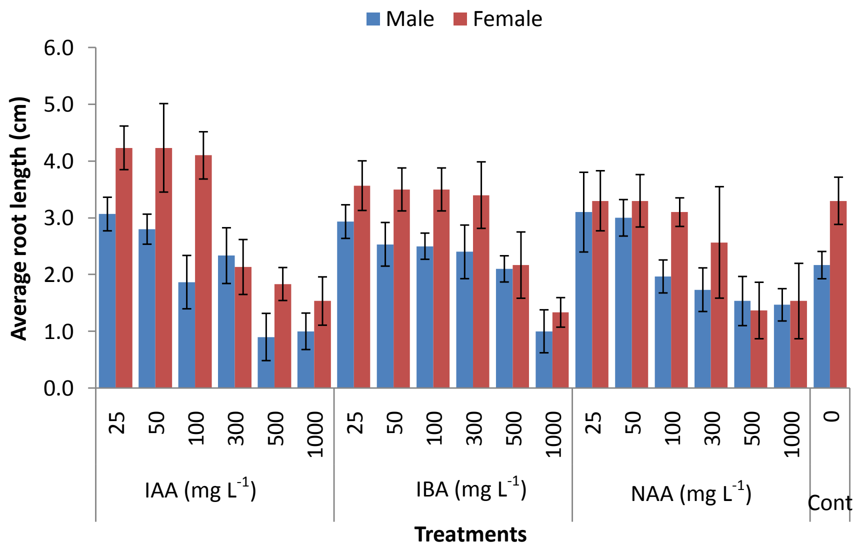

Figure 3. Effect of different auxin treatments against control on elongation of root length of male and female plant cuttings after 90 days.

Table 1. Statistical data representing the overall significant and non-significant different (P<0.05) for different growth parameters of male and female cuttings.

Parameter	Factor	Auxin					
		IAA		IBA		NAA	
		P-value	F crit	P-value	F crit	P-value	F crit
Rooting percentage	Within concentration	0.00*	5.05	0.00*	5.05	0.01*	5.05
	Within sex	0.88^{ns}	6.61	0.04*	6.61	0.25^{ns}	6.61
Root/shoot	Within concentration	0.01*	5.05	0.42^{ns}	5.05	0.02*	5.05
	Within sex	0.00*	6.61	0.04*	6.61	0.00*	6.61
Root length	Within concentration	0.03*	5.05	0.00*	5.05	0.01*	5.05
	Within sex	0.03*	6.61	0.01*	6.61	0.11^{ns}	6.61

*Significant different; ^{ns} non-significant. IAA, Indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, naphthalene acetic acid.

Sex of donor plant has an important effect on rooting of *H. salicifolia* cuttings. The effect of sexual differences on propagation was also observed in several other dioecious plant species like *Taxus wallichiana, Taxus brevifolia,* and *Taxus cuspidate* (Davidson and Olney, 1964; Mitchell, 1997; Nandi et al., 1996; Kaul, 2008). The same results have been reported for many plant species (Sharma and Aier, 1989; Zeng et al., 2005; Guo et al., 2009).

The Dunnent test between control and treated cuttings clearly indicated the importance of auxin application for enhancing and stimulating the rooting traits of stem cuttings. It has been repeatedly confirmed that auxin is required for adventitious root formation on stems and that the divisions of the first root initials are dependent on exogenous and endogenous levels of auxins (Ludwig-Muller, 2000; Kochhar et al., 2005). However, the promoting effect varied with auxin concentrations and types of auxin applied.

In the present study, the lower concentration of auxin did substantially produce better rooting results than the higher concentration of auxin. IBA 50 mg L^{-1} produced the best rooting results rather than other higher concentration of auxins and hence was recommended for vegetative propagation of *H. salicifolia*. This inhibitory effect caused by high exogenous auxin also occurred in other plants species such as peach (Tworkoski and Takeda, 2009) and Paeonia (Guo et al., 2009). The study is also strengthened by the Garonovich (2003), who recommended treatment of *Hippophae* cuttings with IBA (100 mg/L), IBA (50 mg/L) and NAA (50 mg/L) for 16 h.

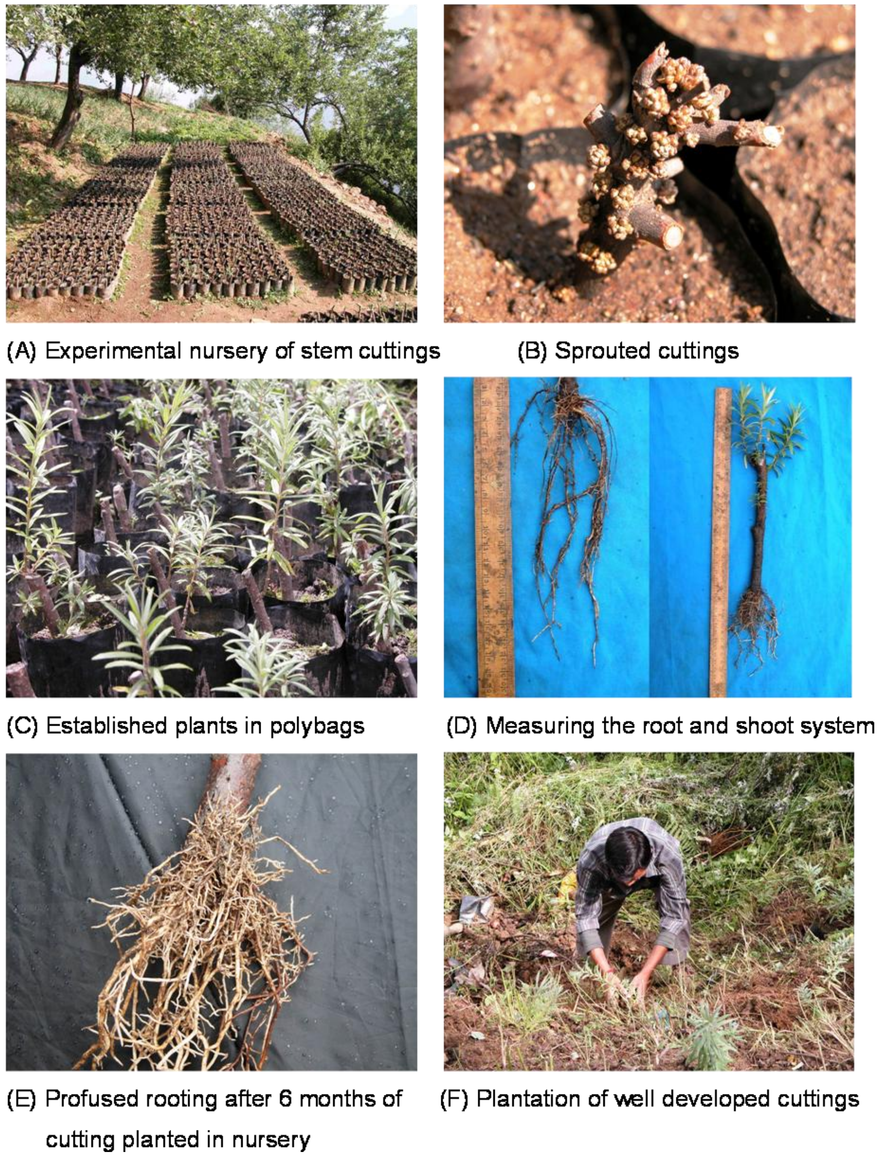

Figure 4. (A-F) Showing details about vegetative propagation of *Hippophae salicifolia.*

Most of the tree species rooting ability of cuttings has been observed from apical to basal part of the shoots which has been attributed to accumulation of carbohydrates at the base of shoot (Hartmann et al., 1997). Based on this fact and several studies by Hartmann et al. (1990), Wassner and Ravetta (2000) and Guo et al. (2009), we have also collected cuttings from the young shoots of *H. salicifolia* for obtaining better rooting traits. Such effects on rooting may be caused by variation in the physiological status of the cutting tissues on stock plants resulting in occurrence of gradients in the cellular activity or in the level of assimilates or growth regulators or in the level of lignifications (Hartmann et al., 1990, 1997).

To enhance the rate of adventitious roots development, auxin application has been found to increase the number of roots initiated per rooted cuttings in a variety of plant species (Mesen et al., 1997; Palanisamy et al., 1998; Kesari et al., 2008). The result obtained for number of root per rooted cuttings in the present experiments was not significant ($p>0.05$) for both male and female cuttings (Figure 2 and Table 1). It was observed that the number of roots per rooted cuttings was found maximum if treated with NAA 25 mg L^{-1} while, the minimum number was observed in the 500 and 1000 mg L^{-1} of IAA (Figure 2). On an average there were 3 to 5 roots formed in a shoot. Similar to earlier study by Houle and Babeux (1998) on *Salix planifolia* the number of roots per rooted female cutting of *H. salicifoila* was found higher as compared to male cuttings. Shuhua et al. (1989) also measured the same number of average roots in *H. salicifolia*.

The cuttings treated with different concentrations of IAA, IBA and NAA showed the significant differences ($p<0.05$) for the root length of male and female cuttings (Table 1). The results reveal that the IBA 50 mg L^{-1} served as a best medium to stimulate and enhance root length. The overall results showed 3.5 to 5 cm mean root length for 90 days old stem cuttings of the target species. Shuhua et al. (1989) observed an average of 3.6 cm long root for the cuttings collected from the base part of Seabuckthorn plant. The earlier work indicated that after a period of 7 months of planting, the root length increased up to more than 15 cm long under various treatments / concentrations of IBA (Sankhyan et al., 2003).

The result of the present study also showed profuse rooting with maximum of 19 cm elongated root after 6 months of plantation (Figure 4D).

Conclusion

The study provides significant information towards development of a simple and cost-effective technique for large scale propagation, cultivation, afforestation of elite genotype under *ex-situ* conditions. The findings of the present study revealed that the lower concentration of auxins provide better results of rooting traits than higher concentration and recommends the application of IBA 50 mg L^{-1} in *H. salicifolia*. Since, it is a potential multipurpose plant having a huge economic potential, the present study also provides a considerable guidance towards the future domestication of this species for poverty alleviation through orchard development that generate income for poor subsistence farmers in Indian Himalayan Region.

REFERENCES

Ahmad SD, Kamal M (2002). Morpho-molecular characterization of local genotypes of *Hippophae rhamnoides* L. ssp *turkestanica* a multipurpose plant from northern areas of Pakistan, online. J. Bio. Sci., 2(5): 351-354. Chen T (1988). Studies of the biochemical composition of *Hippophae* and its quality assessment in Gansu Province. In *Hippophae*, 1: 19-26.

Davidson H, Olney A (1964). Clonal and sexual differences in the propagation of *Taxus* cuttings. Combined proceeding international plant propagation society, 14: 156-160.

Dhyani D (2007). Exploration, multiplication, identification of elite population of *Hippophae rhamnoides* (Seabuckthorn) for sustainable rural development of higher Himalayan region of Uttaranchal, Ph.D. thesis, H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, India, pp. 173.

Dhyani D, Maikhuri RK, Rao KS, Kumar L, Purohit VK, Sundriyal M, Saxena K (2007). Basic Nutritional Attributes of *Hippophae rhamnoides* (Seabuckthorn) Populations from Central Himalaya, India. Curr. Sci., 92(8): 1148-1152.

Fuheng Wu (1991). Seabuckthorn medicine in Russia. Seabuckthorn, 4 (2): 38-41.

Garonovich IM (2003). Introduction of *Hippophae* L. in Belarus. In: Seabuckthorn- A multipurpose Plant. In: Singh, V. (Eds.), Botany, Harvesting and Processing Technologies I, Indus Pub. Co. New Delhi, pp. 135-151.

Gayle E (2008). Sea Buckthorn, Herb. Gram. American Botanical Council, 78: 1-2.

Guo XF, Fu XiLing, Zangh D, Ma Y (2009). Effect of auxin treatments, cuttings' collection date and initial characteristics on Paeonia 'Yang Fei Chu Yu' cutting propagation. Sci. Hortic., 119: 177-181

Hartmann HT, Kester DE, Davies F, Geneve RL (1997). Plant propagation: Principles and Practices. 6th Edn. Prentice Hall of India Private Limited, New Delhi, pp. 276-328.

Hartmann HT, Kester DE, Davies FT (1990). Plant propagation, Principles and Practices. Hartmann H. T., Kester D. E., Davies F. T. (Eds.). Prentice Hall, Englewood Cliffs, pp. 647.

Houle G, Babeux P (1998). The effects of collection date, IBA, plant gender, nutrient availability, and rooting volume on adventitious root and lateral shoot formation by *Salix planifolia* stem cuttings from the Ungava Bay area (Quebec, Canada). Can. J. Bot., 76(10): 1687–1692.

Jain SK (1991). Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications, New Delhi, India, pp. 311.

Kaul K (2008). Variation in rooting behavior of stem cuttings in relation to their origin in *Taxus wallichiana* Zucc. New For., 36: 217-224.

Kesari V, Krishnamachari A, Rangan L (2008). Effect of auxin on adventitious rooting from stem cuttings of candidate plus tree *Pongamia pinnata* (L.), a potential biodiesel plant. Trees, DOI 10. 1007/s00468-008-0304-x

Kochhar VK, Singh SP, Katiyar RS, Pushpangadan P (2005). Differential rooting and sprouting behavior of two *Jatropha* species and associated physiological and biochemical changes. Curr. Sci., 89(6): 936-939.

Koelz WN (1979). Notes on the ethnobotany of Lahaul, a provinence of the Punjab. Q. J. Crude Drug Res., 17: 1-56.

Lebeda AF (2005). Processing Methodologies of Seabuckthorn (*Hippophae rhamnoides* L.) Food Products. Seabuckthorn (Hippophae L.): A multipurpose wonder plant. In: Singh, V. (Eds.). Daya publishing house, New Delhi, India, 2: 533-541.

Ludwig-Muller J (2000). Indole-3-butyric acid in plant growth and development. Plant Growth Regul., 32: 219-230.

Mesen F, Newton AC, Leakey RRB (1997). Vegetative propagation of *Cordial allidora* (Ruiz and Pavon) Oken: the effects of IBA concentration, propagation medium and cutting origin. For. Ecol. Manage. 92: 45-54.

Mingyu Xu (1994). Anticancer effects of and direction of research on *Hippophae*. *Hippophae*, 7: 41-43.

Mitchell AK (1997). Propagation and growth of pacific Yew (*Taxus brevifolia* Nutt) cuttings. Northwest Sci,. 71(1): 56-62.

Nandi SK, Palini LMS, Rikhari HC (1996). Chemical induction of adventitious root formation in *Taxus baccata* cuttings. Plant Growth Regul., 19: 117-122.

Palanisamy K, Ansari SA, Pramod K, Gupta BN (1998). Adventitious rooting in shoot cuttings of *Azardirachta indica* and *Pongamia pinnata*. New For., 16: 81-88.

Raffo A, Paoletti F, Antonelli M (2004). Changes in sugar, organic acid,flavonol and carotenoid composition during ripening of berries of three Seabuckthorn (*Hippophae rhamnoides* L.) cultivars. Eur. Food Res. Technol., 219: 360–368.

Rongsen Lu (1992). Seabuckthorn: A Multipurpose Plant Species for Fragile Mountains. ICIMOD Occasional Paper No. 20, Kathmandou, Nepal, pp. 62.

Sabir SM, Maqsood H, Hayat I, Khan MQ and Khaliq A (2005). Elemental and nutritional analysis of Seabuckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) berries of Pakistani origin. J. Med. Food, 8: 518–522.

Sankhyan HP, Sehgal RN, Bhrot NP (2003). Macro-propagation of seabuckthorn (*Hippophae rhamnoides* L.) through shoot cuttings in cold deserts of Himachal Pradesh. J. Hill Res., 16(1): 54-56.

Sharma SD, Aier NB (1989). Seasonal rooing behavior of cuttings of plum cultivars as influenced by IBA treatments. Sci. Horti. 100, 251-255.

Shuhua H, Zhixiang Z, Xin Z (1989). Studies on the culture technique of hardwood cuttings of Seabuckthorn. In: Proceeding of International symposium on Seabuckthorn, , Xian, China, pp. 217-229.

Thomas SC Li (2002). Product Development of Seabuckthorn. Reprinted from: Trends in new crops and new uses. J. Janick and A. Whipkey (Eds.). ASHS press, Alexandria, VA, pp.393-398.

Tiitinen KM, Yang B, Haraldsson GG, Jonsdottir S and Kallio HP (2006). Fast analysis of sugars, fruit acids, and vitamin C in sea buckthorn (*Hippophae rhamnoides* L.) varieties. J. Agric. Food Chem., 54: 2508-2513.

Tworkoski T, Takeda F (2009). Rooting response of shoot cuttings from three peach growth habits. Sci. Hortic.. 115: 98-100.

Venugopalan VV, Ranjit A, Sarinkumar K and Arumughan (2005). A Green technology for the Integrated processing of fresh Seabuckthorn Berries. *Seabuckthorn (Hippophae* L.): *A multipurpose wonder plant*, Vol. 2 (V. Singh, Editor-in-chief, 2005), Daya publishing house, New Delhi, India, pp. 522-532.

Wassner D, Ravetta D (2000). Vegetative propagation of *Grindelia chiloensis* (Asreraceae). Ind. Crop. Prod., 11: 7-10.

Xing J, Yang B, Dong Y, Wang B, Wang J, Kallio H (2002). Effects of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. Fitoterapia, 73: 644-650.

Zeb A (2004). Important therapeutic uses of Sea Buckthorn (*Hippophae*): a review. J. Biol. Sci., 4: 687-693.

Zeng DX, Yin WL, Wang YH, Zhao XQ, Wang HF (2005). Propagation with etiolated softwood cuttings of five dwarf cultivars of Chinese tree peony. (in Chinese, with English abstract). Acta Hortic. Sin., 32, 725-728.