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Propagation Of *Hippophae Salicifolia* D.Don Through Air-Layering

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Abstract: *Hippophae salicifolia* (Seabuckthorn) belongs to family Eleagnaceae. The plant is undergoing apocalyptic decline due to which it has been listed as a threatened species. Measures should be taken at an earliest to conserve this species. Natural Propagation through seeds and seed establishment is rare and problematic in this species. To overcome such problem, an attempt has been made to conserve this threatened plant species through Air-layering. Two auxins namely IBA (Indole Butyric acid) and IAA (Indole acetic acid) in different concentration were used in spring and rainy season to induce rooting in stem cuttings. IBA at 50 ppm in spring season showed maximum number of roots and root length as 9±1 and 7.92±1.22 cm respectively. In rainy season IBA at 50 ppm showed maximum number of roots 24±5.47 with root length 9.88±0.342 cm. Air-layered branched treated with IBA 50 ppm had shown maximum number of roots as well as root length in rainy season. The well rooted branches were transferred and planted to polybags for hardening. Present study showed vegetative propagation through Air-layering when propagation through seeds in nature is scare. This method is cost-effective with large scale production rate in relatively shorter period of time.
Keywords: - Propagation; endangered; Indole-3-Butyric acid; Indole Acetic acid; Hardening

Introduction

Hippophae salicifolia (Elegenaceae), a cold temperate Himalayan deciduous plant, grows widely in India in the Leh and Kargil district of Jammu and Kashmir, Lahaul and sptti of Himanchal Pradesh and Dibang valley of Arunachal Pradesh. It is widely distributed in Gangotri, Harsil, Sukhi, Badrinath, Yamnoutri, Gaurikund and Harki-dun areas of Uttarakhand.The species has been Listed in Threatened category. The natural occurrence of the species can be found in the landslide and roadside sloppy area. It can be identified with the thorny appearance, green lanceolate leaves and orange-yellowish berries. It is a dioecious plant. Male and female are different. Male can be identified by the flower in axillary cluster form while female flower remains solitary. Fruits are highly sour in taste. The plant flowers in the month of March-April and bears fruits in the month of October – December (Sharma and Singh, 2017). The plant has high nutritive, medical and ecological benefit. It is the only plant in the Himalayan region that overcome poverty and hunger prevailing in mountain region during cold and thunder. Local communities' economy is dependent on this plant as they use this plant for fuelwood, fodder, bio-fences, maintain irrigation channel and also used as quality timber production. The plant is also used for the treatment of burns, scalds, gastric ulcer, oral mucosities, rectal mucosities, cervical erosion, radiation damage, skin ulcer, for the treatment for cancer, cardiovascular system and immune system. The plant is also used in skin health as a cosmetic. The essential fatty acid content in Seabuckthorn oil ranges from 80% to 95%. Among the carotene found are alfa and beta carotene, lycopene, cryptoxanthin, zeaxanthin, taraxanthin and phytofluin. Phytosterol includes beta-sistorol, beta-amiorl and erithrosterol (Lu, 1992).

As being threatened species, long term survival can be accomplished by proper propagation techniques. The plant is mainly

propagated through seeds. Consequently, seedlings are not identified and they cannot be distinguished whether they are male or female. Seed dormancy and poor germination hinder large scale multiplication of Seabuckthorn (Pant et al.2014). Moreover, desirable characteristics that are common to the parents may be lost. Other methods of propagation are stem cuttings, layering and through suckers (Li and Schroeder, 1996). Hardwood and softwood stem cuttings is simple procedure, rooting rate are higher but requires special skills, excessive material, proper care and huge labour work. In case of suckers, it is simple and in expensive technique but have poor root mass and may be susceptible to transplant shock (Li and Beveridge, 2003). Micropropagation using tissue culture technique requires high technology and well training, which is not available in places where Seabuckthorn can be grown (Dolkar et al, 2016). Other method of vegetative propagation is through Air-layering. It is alternative technique for propagation of elite plant species. Large scale production can be achieved through this in-vivo technique. It is inexpensive, require less labour and care. Moreover, as compared to other methods of propagation this in-vivo method is less prone to biotic and abiotic stress, where stem cuttings are more prone towards stress and diseases (Sarkar et al. 2022). Currently, there is no published report on standardisation of any propagation method of *Hippophae salicifolia* using Air-layering method. In the present study, experiments have been conducted to examine the rooting behaviour of air-layered stem of *Hippophae salicifolia*. Different doses of auxins namely IAA and IBA were given in different season of the year to examined the effect onto rooting behaviour.

Material And Methods

1. Experimental Design and Treatment

Young random seedlings from natural population of *H.salicifolia* in Gangotri (Figure 1 a-b) and Hanumanchatti (Badrinath) were collected and grown in polyhouse of Research centre. The air layering experiments were conducted to study rooting behaviour of *Hippophae salicifolia* with different concentration IBA and IAA. Stem cuttings from true plants were taken for experiments. Two seasons Spring and Rainy season were selected to conduct experiment on Hard and softwood stems. The present study was undertaken in the month of February- May (Spring) and July- August (Rainy season). The different concentration of IBA and IAA used were10 ppm to 50 ppm. Two control sets were raised as C1 and C2, treated without and with distilled water respectively. Different Auxin concentration were prepared and mixed with Talcum powder to make a paste. Stems of actively growing branches 15-20cm in length and 1-2cm in diameter with 15-20 leaves on lower canopy were selected for exogenous hormonal treatment. Below nodal region, circular strip of bark was grilled with knife without injuring underlying wood and tissues. Talcum paste was used to cover the grilled exposed surface. The paste was further covered with 0.1% aqueous solution of sphagnum moss. Transparent polythene or black polythene was tied at both the end to cover sphagnum moss (Figure 1 c-d)

2. Data collection

Undisturbed air-layered branches allow root initiation. Watering after every 2 days was essential during spring rather than in rainy season. Root initiation was visible within transparent polythene. After 10 weeks, fully raised roots were observed. The rooted branches were detached from parent plant using secateur. The roots were washed with distilled water. Root number, root length was calculated and measured. Rooted branches were then transferred to polythene bags containing soil, sand and manure for acclimatization and hardening.

3. Stastistical Analysis of Data

Mean values for root number and root length was calculated and were subjected to Anaylsis of variance (ANOVA) at $p<0.05$ level.

Result And Discussion

Air-layered branches of *H.salicifolia*, treated with exogenous Auxins were studied. As compared to control, root initiation was faster in Air-layered branches treated with auxins. Within 10 weeks, IAA 50 ppm in spring had shown 7.6±0.547 roots with 5.6±0.938 cm root length (Figure 1 e, Table 1) IBA at 50 ppm in spring season showed maximum number of roots as 9±1 with root length of 7.922±1.22 cm (Figure 1 f, Table 2). During rainy season IAA 50 ppm had shown 7.6±0.547 number of roots with 8.8±0.707 cm root length (Figure 1 g, Table 3). AT 50 ppm IBA, maximum number of roots was 24±5.477 with a root length of 9.88±0.342 (Figure 1 h, Table 4).

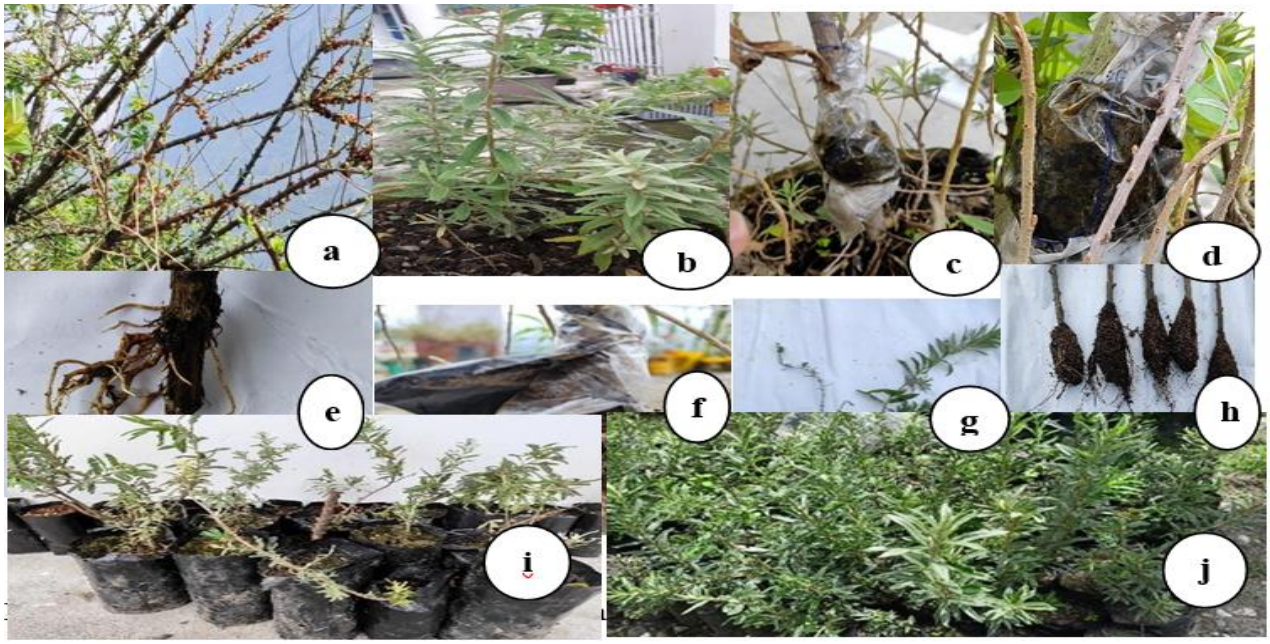

Fig. 1 Propagation of *H.salicifolia* through air-layering;(a-b) natural population of *H.salicifolia*; (c-d) covering grilled portion with sphagnum and transparent polythene; (e) rooting in branches treated with IAA 50 ppm in spring; (f) rooting in branches treated with IAA 50 ppm in rainy season; (g) rooting in branches treated with IBA 50 ppm in spring; (h) rooting in branches treated with IBA 50 ppm in rainy season; (i-j) rooted.

Table 1: Effects of IAA onto rooting behaviour of Air-layered branches in Spring season.

Treatments	Mean Roots number	Mean Root length
C1	2.6±0.547	1.7±0.565
C2	3.4±0.894	2.28±0.148
10 ppm	3.8±1.303	2.56±0.545
20 ppm	4.2±1.303	2.96±0.427
30 ppm	4.2±1.095	3.06±0.498
40 ppm	5.8±0.447	4.22±0.130
50 ppm	7.6±0.547	5.6±0.938

Table 2: Effects of IBA onto rooting behaviour of Air layered branches in Spring season.

Treatments	Mean Roots	Mean Root length
C1	4.4±0.894	1.34±0.114
C2	4.6±0.547	1.76±0.507
10 ppm	5.6±0.547	2.5±0.636
20 ppm	5.8±0.447	1.8±0.509
30 ppm	5.2±1.095	2.82±0.491

40 ppm	6.2±0.837	3.82±0.571
50 ppm	9±1	7.92±1.22

Table 3: Effect of IAA onto rooting behaviour of Air-layered branches in Rainy season.

Treatments	Mean Roots number	Mean Root length
C1	2±0.7071	1.72±0.460
C2	2.8±0.836	2.42±0.661
10 ppm	4±0.707	2.18±0.563
20 ppm	4.4±0.894	4.36±1.016
30 ppm	5.8±0.836	6.02±0.540
40 ppm	6±0.707	6.76±0.134
50 ppm	7.6±0.547	8.8±0.707

Table 4: Effect of IBA onto rooting behaviour of Air-layering branches in Rainy season.

Treatments	Mean Roots	Mean Root length
C1	14.8±0.836	3.94±1.105
C2	16.4±0.547	5.68±0.739
10 ppm	16±0.707	4.74±1.514
20 ppm	16.4±1.341	5.08±1.215
30 ppm	17.2±1.095	5.3±1.153
40 ppm	17.8±0.445	6.68±0.743
50 ppm	24±5.477	9.88±0.342

Air-layered branches were transferred to polybags containing soil, sand and manure in the ratio 1:1:1 for hardening and acclimatisation (Figure 1 i-j). The air-layered plant in polybags were well acclimatised with 100% survival rate.

In *Blighia sapida*, 3500 ppm of IBA was most effective for root initiation in air-layering stem (Maurya et al. 2013). However, lower concentration of Auxin is reported effective for root initiation in *Quercus serrata* (Srivastava et al. 2000). In present study also IBA at lower concentration is efficient for root initiation in Seabuckthorn. As number of roots and root length obtained due to effect of IBA was greater as compared to IAA. For critically endangered species like *Fagraea auriculatum*, treatment with IBA is proved to be best for rooting in Air-layered branches (Yeo et al. 2011). Relatively, Air-layered branches in *H.salicifolia* treated with IAA induces late rooting after 20 weeks as compared to IBA rooting within 10 weeks. Minimum time for root initiation was observed with the treatment of IBA in *Lasiococca comberi* (Kamila PK and Panda PC, 2018). Metabolism of two auxins IAA and IBA causes difference in rooting behaviour in *Grevillea* (Proteaceae) (Krisantini et al. 2006). Endogenous and exogenous auxin interaction is also important for root induction in *Jatropha curcas* and *Jatropha glandulifera* (Kochhar et al. 2005). In various classes of growth regulators, IBA have significant role in the level of root-initiating inhibitor and rising root inducing co-factor that leads to early rooting. (Nanda, 1975). Rainy season was efficient for root initiation in Seabuckthorn stem cuttings as active growth initiates in the advent of rainy season and continues till October (Agarwal et al. 2015).

Air-layering using exogenous hormones proved to be efficient technique. Mass large scale production with genetically identified plant can be achieved through this process. Air-layering ensures large-scale production by avoiding loss of genetic diversity. Even mass scale production can be achieved if applied to small local population. It is one of the best techniques to conserve plant species that are undergoing threat of being extinct.

Conclusion

Present study suggests vegetative propagation through air-layering in *H.salicifolia* for large-scale production of similar genotype. It is one of the best methods of propagation for elite genotype as compared to other alternative methods. Induction of rooting within 10 weeks in *H.salicifolia* with 50 ppm IBA concentration proves to be best for root initiation in Seabuckthorn with maximum root

number 24±5.47 and maximum root length 9.88±0.342. Lower concentration of IBA is efficient for root initiation. Rainy season is proved to be best for root induction. Large scale production of Seabuckthorn in minimum time interval can be achieved using this process.

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