

# Standardization of Agro-techniques of *Hippophae salicifolia* D. Don (Seabuckthorn) of Sikkim Himalayas

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## SUMMARY

From the present study on *Hippophae salicifolia* D. Don growing in Sikkim and north-east Indian Himalayas, it was observed that the plant is not very big in size, but trees up to 15 meters have been recorded. The ripe fruit is 4-6 mm length and 3-6 mm in width with orange red colour contained various compounds like vitamin C (300-1600 mg/100 g), vitamin E (200 mg/100 g), lycopene, flavonoids (0.2 per cent in juice), amino acid, etc. The study on germination of seeds in different soil media was done; fertile and brown soil media gave good result. Rooting and callus formation using growth regulators like IBA, NAA and IAA in different concentrations (ppm) showed interesting growth results, in which IBA (55-60 ppm) showed more callus formation and rooting within three months. Studies carried out in different chemicals for germination of seeds of seabuckthorn in which GA3 (700 ppm) treated seeds germinated in 8 days as compared to 17 days in water alone (control). Further response of hormones in root growth showed that IAA at 100 ppm showed mean root length of 9.4 cm. This type of study for plant with high economic potential, can lead to the better understanding and management in the natural condition.

**Keywords:** *Hippophae salicifolia*, Dalle-chuk, Lepcha, Nepali, Bhutia, seabuckthorn, Hormones, Soil, Modulated, Riverine, IBA, NAA and IAA.

## INTRODUCTION

Sikkim, Himalayan state of India is situated in north-east India at 28°07'48" and 27°04'46" North latitude and 88°00'58" and 88°55'25" East longitude with a total area of 7096 sq km (Anonymous, 1982) and has an average distance of 64 km from east-west and 114 km from north-south. This tiny state has immense biological diversity without any of its parallels elsewhere in the world (Sharma et al. 1995), with elevation ranging from 300 m to 8500 m asl (Basistha et al., 2001).

*Hippophae salicifolia* (Seabuckthorn) locally known as Tarobo (Bhutia), Ree-singri (Lepcha) and Ghanguru/Achuk or Dale-chuk (Nepali) is a fascinating plant species known and used by humans for centuries (Dhakal, 2001).

*Hippophae salicifolia* belongs to family *Elaeagnaceae*, is a dioecious, wind pollinated plant with more than 60 years of lifespan (Rongsen, 1992; Jeppson et al, 1999; Nepal et al, 2001). Seabuckthorn (*Hippophae L.*) is a multipurpose and economic plant of the dry temperate Himalayas and is a native of European countries, China and India (Singh, 1998). The species is known for fixing atmospheric nitrogen into the soil through symbiotic relationships with root inhabiting micro-organism the Frankia by formation of root nodules (Andreeva et al, 1982). The roots of plant are also known for better ecological advantages, such as soil binding and erosion control at fragile hilly slopes (Rongsen, 1992; Singh et al, 1995; Singh, 1998). Apart from its ecological value, it has medicinal value also (Dhakal, 2001).

In cold deserts of India, the plant starts its foliage in April and bears flowers in monsoon and fruiting starts in August and retains till April (Singh, 1998). But in Sikkim, it was observed that flowering started in March and fruiting in April which ripens in October-November and retained till March. Since, its root system is well developed; the plant could hold soil even in fragile condition.

Agro-techniques of seabuckthorn through different methods like cuttings, seed propagation (Auazato et al, 1987), layering, etc. have also been done in the past which along with natural habitat shall help to understand the genus closely in the local environment.

In Sikkim, the plants are observed to be growing in the areas of about 500 ha at different slopes and north east aspects in colonies or lone stand of trees (6-10 meters), on the either side riverbanks (riverine) and non river side (non-riverine) areas in and around Zema I, Zema II, Zema III, Puchi and Chaten of Lachen valley and adjoining areas Lachung, in the north district of Sikkim. The plant has been found growing at the altitudes

ranging from 2377 m to 3093 m asl (Table 2.1). The plant for its faster regeneration favoured the landslide areas (new soil) of sun facing slopes and riverbanks with sandy to coarse textured soil (Basistha, 2001; Basistha et al., 2001). Seabuckthorn has good association with other floral species like Populus sp., bamboos, Daphnie, Rhododendron sp., conifers, etc. Its association with *Alnus nepalensis* (also actinorhizal plant) at the lower altitude where seabuckthorn started northwards from that point at an altitude of 2377 m. This reveals variation in climatic features of soil and air at natural orchard of seabuckthorn.

Table 2.1: Geophysical Parameters at Natural Habitat of Seabuckthorn in Sikkim

Parameters	Natural Habitat			Experimental Field (at Tadong) (1424 m)
	Low Altitude (2377 m)	Medium Altitude (2600 m)	High Altitude (3093 m)	
Aerial temperature	23°C	19°C	19°C	26°C
Humidity	89 per cent	92 per cent	17 per cent	92 per cent
Soil pH	6.6	6.6	6.8	6.6
Soil temp.	17.8°C	18°C	13.9°C	19.3°C
Soil moisture	65 per cent	70 per cent	35 per cent	70 per cent
Soil colour	Brown	Brown	Brown	Brownish-Black
Aspect	North	South-East	South-East	South-East
Management	Natural	Natural	Natural	-
Site cover	20-40	20-40	20-40	-

The local people use the plant as cheap source of firewood. It was learnt from the locals that the wood of seabuckthorn bums with almost blue flame and gives a good heat with minimum of smoke and ash. People prepare jam and pickles out of the fruits, which, they said, is good for digestion. Local women weavers, craftsmen use the juice from the fruit as dyeing ink for their traditional blankets, bedcovers and sweaters, etc.

## MATERIALS AND METHODS

Frequent surveys on occurrence of the plant and morphological studies were carried out in 1999- 2000 in different seasons, i.e. flowering season (February-March), fruit ripening season (November- February) and vegetative growth season (April-July) and different physico-climatic conditions and data were recorded. In the month of June 2000, the specimens of the plant parts were collected from different areas and herbarium was prepared for further studies. The area was divided into three categories based on altitude viz., low (2377 m), medium (2600 m) and high (3093 m) altitude of the *Hippophae salicifolia* growing area. A study on propagation of the plant was carried out at natural habitat and fields of Sikkim State Council of Science and Technology at 1424 m.

The fruits were also collected, both pulp and dried fruits were sent to Central Food Technological and Research Institute (CFTRI), an ISO 9001 and 14001 organization, which is a premier Institute in India, located at Mysore, Karnataka.

In-situ geophysical chemical studies analyzing different parameters were recorded from the field of natural orchard of *Hippophae salicifolia* at Lachen and Lachung valley using digital thermometer (Multi thermometer, CE make, humidity meter (Model LR6Mignon AA, England make), soil pH and moisture were determined with on the spot tester soil pH and moisture meter (Takemura, Japan make, model DM-15) and the soil temperature with a soil thermometer. The recorded data was interpreted with that of the trial field and other similar habitats of the species (Table 2.1).

Both hardwood and softwood cuttings were taken from the matured trees (approximately 5-10 years old) from the natural orchard in an appropriate manner in the month of January and were later waxed and pretreated with Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and Indole acetic acid (IAA) at different concentrations for overnight. The treated cuttings were later transferred and planted into separate nursery bed of 0.5 x 0.5 sq m with in 15 cm spacing. The soil composition was maintained as sand: soil: compost at 70:20:10. Watering and manuring were done as per the climatic situations. Twenty cuttings were used for the treatment with each concentration of 50 ppm, 55 ppm and 60 ppm of IBA; 300 ppm, 350 ppm and 400 ppm of NAA; and 50 ppm, 100 ppm and 200 ppm of IAA. The result was recorded and tabulated accordingly.

Air layering was carried out simultaneously by taking about 20 numbers of 1-2 years old branches at sun facing side amongst 5-10 years old trees and using local moss and soil mixture supported by transparent polythene strips during the month of April, and collected in the month of late July and planted in the experimental field at 1424 m.

Propagation was also studied using freshly collected seeds from the well ripened fruits collected from the natural habitat in the month of December. These seeds were dried for 5 days and washed properly with water and soaked for 5 hours. The seeds were then imbibed. The germination was done in raised beds with different soil types/soil compositions. The imbibed seed germination was also done in Petridishes treated with varying concentrations of 50 ppm, 55 ppm and 60 ppm of IBA; 300 ppm, 350 ppm and 400 ppm of NAA; and 50 ppm, 100 ppm and 200 ppm of LA A and were studied in the laboratory (Tadong, Gangtok) at room temperature. These were later transferred to 10" polybags for further growth. The mean root length data related to germination in each of the concentrations pertaining to growth, rooting and nodulations, etc., were recorded and analyzed.

## RESULTS

### Morphology

*Hippophae salicifolia* usually a bushy tree measured up to 15-18 m in height, in some area of Zema II and in the adjoining river bank with presence of heavy branches and a big foliage canopy. In Lachen near the monastery, male and female trees nearly 15-20 m measuring with girth of 2.5 m has been standing.

The leaves measured up to 72-9.7 cm in length and 1.1-2.0 cm width with 3-5 mm stalk length, linear, lanceolate, dorsi-ventral with and lower side covered with silvery satellite scales and pubescent hairs.

The male and female flowers were markedly different in their appearance, as male floral buds consisted of 4 to 6 flowers in cluster, while the female floral buds possess only one flower and rarely, two in the current season growth and in the older branches. Both male and female flowers had no petals, and possess four stamens and one pistil with conspicuous single ovary in male and female flowers, respectively. Often, floral buds were found mixed with the vegetative buds and were rarely in pure.

The roots of the plant were seen mostly confined around to 1-1.5 m of the soil with plant height of 1-2 m. A long taproot was found in most cases, penetrating down the surface up to 0.5 to 1m in the non- riverine areas. Approximately, two-year old plant was measured with root length of 1m at Zema III (3093 m asl).

The ripe fruits of seabuckthorn in its habitat were orange or reddish yellow in colour and were measured 4 to 6 mm in length and 3 to 6 mm in width with small black pustules at maturity, over the outer covering of the fruit with a notch. The seeds of the plant were brown to dark-brown in colour, 3 to 4 mm in size and the test weight of 0.97 g. The roots were found restricted to comparatively less depth in the soil with maximum percentage of humus and black soil\*

### Habitat

From Table 2.1, it is revealed that *Hippophae salicifolia* grows naturally in fragile slopes with high risks of landslides and flash washes, ranging from 2377 m asl to 3093 m asl. The plant survived and grew well in the experimental plot situated at Tadong, Gangtok at an altitude of 1424 m asl. During the season, not much variation was observed in the temperature of natural habitat at medium and high altitudes (19 °C), which may be due to the gentle rise in slopes of the study area. The soil pH at the natural habitat found to be varying from 6.6 to 6.8 and the moisture level was in the range of 35 to 70 per cent. The plant was happily growing in almost open areas in brown soils facing south east aspect with plenty of natural light.

### Germination Performance

The Table 2.2 presents the details on germination and growth pattern of seedlings in different soil based media. It shows that germination in artificial media (Kel-perlite) and black soil took 17 and 18 days, respectively, as compared to faster germination in sandy soil (13 days), which may be due to more aeration as compared to the other two soils used. 50 per cent germination was seen better in brown soil which took 21 days. 85-90 per cent germination was seen in perlite soil with better average growth (1.9 cm/month). Perlite being an artificial media may not be advisable for the rural conditions and it can be concluded that brown soil may be better for the germination and growth of *Hippophae salicifolia* seeds. Sandy soils may be better for initial growth but may not suffice the nutrients for later growth and development.

Table 2.2: Germination Performance of Hippophae Seeds in different Soil Based Media at Laboratory Field, 4700 ft asl

Soil Type	Days to Initial Germination	Days to 50 per cent Germination	Total Germination (per cent)	Growth per Month (Average, cm)
Black soil	18	26	40	1.9
Brown soil	17	21	80-85	1.7
Perlite	17	21	85-90	1.9
Sandy soil	13	22	80-85	1.8

## Vegetative Propagation with Growth Treatment

Table 2.3 shows that IBA at 60 ppm is better for root development (45 per cent) with a significant root length (31.5 per cent) in comparison to the control and other treatments. The callus formation (60 per cent) and mean root length (9.4 cm) was observed to be higher in IAA at 100 ppm treated cuttings. Among the different concentrations of N A A, 300 ppm N A A gave the good results showing 35 per cent success in callus formation and 15 per cent success in root formation with mean root length of 2.5 cm.

Table 2.3: Response of Growth Hormones in Successful Formation of Callus and Rooting in Seabuckthorn Cuttings (90 days after planting)

Treatments (ppm)	Total Rooting		Total Callus Formation		Root Length (cm)	
	(Nos.)	(per cent)	(Nos.)	(per cent)	Total	Mean
IBA 50	7	35	8	40	43	4.8
IBA 55	8	40	9	45	14.6	1.8
IBA 60	9	45	9	45	31.5	3.93
NAA 300	3	15	7	35	7.5	2.5
NAA 350	0	04	20	-	-	-
NAA 400	0	0	4	20	-	-
IAA 50	2	10	10	50	2.2	1.1
IAA100	5	25	12	60	4.7	9.4
IAA 200	3	15	9	45	25.5	8.5
Control (H2O)	5	25	1	5	19.5	3.9

The result from the Table 2.3 shows that cuttings may show good results if treated with 60 ppm IB A in terms of rooting, callus formation and root development as compared to NAA and LA A at the above concentrations.

The average numbers of nodes on the cuttings of *Hippophae salicifolia* were 40 measuring 50 cm and the required length of cuttings for propagation remains 25 cm with 20 buds in each, having average weight of the cuttings as 75 g, the required quantity of the prepared cuttings for the vegetative propagation of seabuckthorn as follows:

Average weight of cutting-75 g.

Crop geometry-2 x 2 m.

Cutting position while planting-vertical.

Cutting for replacement of damage/dead cuttings-20 per cent.

Average number of nodes on the cutting of 50 cm=40.

Type of set/cutting to be used for planting=25 cm.

Average number of damage buds per cuttings=10.

Spacing (row to rows) on planting=200 cm.

Number of cuttings required= 10,000 x 2 x 2 = 2500

Number of cuttings required for replacement=20 per cent of 2500

Total cuttings required =3000

The weight of cuttings required/hectare is 3000 x 75/1000 x 100=225 kg/ha.

Table 2.4: Response of Growth Regulators in Germination of Seeds in Petridish at Room Temperature

Particulars	Control	IBA (ppm)			NAA (ppm)			IAA (ppm)		
	H2O	50	55	60	300	350	400	50	100	200
Mean root length (cm)	3.9	4.8	1.8	3.9	2.5	00	00	1.1	9.4	8.5
Mean root numbers	4.0	4.2	2.9	3.9	3.3	00	00	2.5	5.6	5.3

From the Table 2.4, it was shown that the seeds grown in Petridish containing IBA 50 ppm solution soaked filter paper showed better germination performance in both rooting and shooting. The findings of Tables 2.3 and 2.4 were related with the findings of Donald (1997) who reported that treatment of cuttings of *Pinus ellitti* with 1 per cent IBA for successful root initiation. Similar results were also reported by Tsar'kova (1988) and Rongsen (1992) that 50 ppm IBA presented better initiation of rooting in softwood cuttings of *Hippophae*.

The observations of layering showed that 13 out of total 20 (65 per cent) layerages with successful rooting and foliage growth were collected from Lachen area, and are transplanted in field conditions (1424 m). It was, however, noted that some of the rooted branches were already nodulated with 2-6 lobed nodules, somewhat whitish in colour but intact with the root mass. The layerages had good success rate in the experimental plot without any treatments.

### **Fruit Analysis**

As per the studies carried out from the sample sent to CFTRI, Mysore, the composition of fruits and leaves of seabuckthorn from Sikkim were: Leaves of seabuckthorn contained 11 to 22 per cent of crude protein, 3 to 6 per cent of crude fat and some flavonoids; fruit contains 60-80 per cent juice in which sugar, organic acids and vitamins were rich. The vitamin C content was 200 to 500 mg/100 g, fruits contained 3-5 per cent of pulp oil and 8-18 per cent of seed oil that were rich in unsaturated acids, [3-carotene and vitamin E. In addition, certain content of flavonoids was also found in the residues of fruit; and the fruit may be used for making soft drinks, wines, healthy food, medicines and cosmetics.

### **DISCUSSION**

The present study was conducted in Lachen and Lachung valley of North Sikkim revealed some interesting facts. seabuckthorn plants in riverbank-sandy soil produced higher number of nodules irrespective of the plant size as compared to that from the non-riverine slopes.

In the trial plot, it was observed that the seedlings of even smaller size produced sufficient root mass and formation of nodules were even higher as compared to the plant from natural habitat. Nodulation was even better in the sandy soil with sufficient gravel and organic matters added with cow dung manure. The average number of nodules from 100 seedlings (5-15 cm) was recorded at 12/ plant. The root mass was observed to be poor in case of waterlogged soil and found to be developing better in sandy and coarse sandy soil, which is comparatively lower than the riverbank areas of natural habitat.

It was also noted that the seabuckthorn seedlings grew at greater heights in non-riverine areas of lower altitude and higher altitude but in the case of nodulation, it was reversed. In non-riverine areas of lower altitude, there were fewer nodules per plant as compared to the plants in non-riverine areas of higher altitudes. The roots were found restricted to comparatively lesser depth in the soil with maximum humus and black soil. The heights of the plant were observed to be reducing with the increase of altitude both at riverine and non-riverine areas. The trees up to 14-20 meters can be seen in non-riverine areas of Lachen and seemed to be old natural orchards. The old trees are slowly being replaced by new bushy plants.

### **CONCLUSION**

The present study on agro-techniques for *Hippophae salicifolia* in Sikkim Himalayas reveals that as elsewhere the plant can be artificially explored to several other dry temperate areas of Sikkim with similar climatic conditions. The cultivation of this economic and eco-friendly plant with scientific approaches may fulfill the requirements of high altitude inhabitants. The germination of *Hippophae* seeds using perlite type soil or brown soil media, use of pretreated cuttings for mass propagation for easy and massive plantation programmes and thus, development of greenery at difficult areas, may be taken as viable package of practice for seabuckthorn cultivation in Sikkim and thus, may added to the economy of the marginalized farmers of the remote terrain. The study reveals that if the plants grow well in sandy or coarse sandy soil media or in fields with desirable soil reclamation. In a nutshell, plant creates more biomass in stressed soil and climatic condition. Further studies in details may enable the species to propagate in other localities with varied topographical conditions.

### **ACKNOWLEDGEMENT**

The authors are thankful to DBT, Government of India for Project support, and the Sikkim State Council of Science and Technology, Gangtok.

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