Seabuckthorn (*Hippophae* spp., Family: Elaeagnaceae) is a valuable wild plant species and currently domesticated in various parts of the world. The species is a multi-use plant with its nutritional and medicinal attribute. Seabuckthorn (SBT) has been gaining importance in large scale cultivation in various parts of the globe for its fruits and also due to its wide adaptability that fulfills the afforestation requirements.

### 1.1 Discovery of Seabuckthorn

In an undated publication, Xu Mingyu et al., ([www.seabuckthorn.com](http://www.seabuckthorn.com)) reviewed the historical information on Seabuckthorn. They described that -

“In historical records, Chinese people were the first to use seabuckthorn as a drug. More than a thousand years ago seabuckthorn was recorded in Yue Wang Yao Zhen from the Tang Dynasty and in Sibu Yidian, whose writing was finished in the 8th century. Sibu Yidian is a classical Tibetan medical book in four volumes and contains 158 chapters. Out of these, thirty chapters deal with seabuckthorn medicinal products, mentioning the pharmacological effects on inducing the expectoration, opening the inhibited lung energy, dispersing dampness, tonifying the YIN and strengthening the YANG. More than 60 entries refer to its capacity to strengthen the spleen and the stomach, and to promote blood circulation, to remove blood stasis, and there are 84 prescriptions with seabuckthorn, which come in the form of seven different preparations namely, decoction, powder, pill, medicinal extract, shortbread, ash and tincture. In the 18th century, Sibu Yidian was translated into Mongolian, and later it was translated by European countries for studying and commenting. In ancient Greece, the leaves and young branches of seabuckthorn were used in the fodder to achieve rapid weight gain and a shiny coat. The name of the plant is derived from Latin words ‘Hippo’ which means ‘horse’ and ‘phaos’ which means to ‘shine’. In 1903, Sibu
Yidian was published in Russia in St. Petersburg (Ma Yingcai, 1989). In 1952, seabuckthorn was rediscovered in Tibet and an academic thesis was written on Seabuckthorn by Xu Zhonglu et al., (1956) at Sichuan Medical College, China. The Preliminary Research on the Fruit Juice of Seabuckthorn was published in 1956. Seabuckthorn was listed in the Chinese Pharmacopoeia for the first time in the year 1977”.

The geographical distribution of Seabuckthorn is mainly in Asia but with natural extension through Europe. In India, it grows widely in dry temperate Himalayas, comprising upper regions of Himachal Pradesh, Uttarakhand, Jammu and Kashmir, Sikkim and Arunachal Pradesh (Singh, 2003). In Sikkim Himalayas, a part of North East India, the plant grows on the riverside, torrential slides and sun facing aspects or slopes. The distribution of the species is restricted in Lachen and Lachung valleys of Sikkim at the altitudes ranging from 2377 to 3093 m.a.s.l.

1.2 Taxonomy and morphology of Seabuckthorn

The botanical genus of the seabuckthorn plants is *Hippophae* under the family Elaeagnaceae. The genus includes a total of five species namely - *H. rhamnoides* L., *H. salicifolia* D. Don, *H. neurocarpa* Liu & He, *H. tibetana* Schlecht and *H. goniocarpa*. Out of these three species viz., *H. rhamnoides*, *H. salicifolia* and *H. tibetana* are available in India. Only one species namely, *H. salicifolia* D. Don is reported in Lachen and Lachung Valley of Sikkim (Baisistha et al., 2010). The species *Hippophae salicifolia* D. Don, locally known as Chuk, Tarwa, Tarubo, and Taru, is a deciduous species restricted to the Himalayan region, between 1500–3500 m.a.s.l. (Hooker, 1894; Gaur, 1999).

Seabuckthorn is a dioecious multi-branched, thorny shrub or tree, reaching 2 to 20 ft in height with stout branches forming a round often symmetrical head. It has brown or black rough bark and a thick grayish-green crown (Rousi, 1971). The plant
bears foliage from April to November; flowers during June - July for a week and fruits are formed during mid August to April. Female plant bears red, yellow or orange coloured fruits 1 cm across (Singh, 1998). Leaves are alternate, narrow and lanceolate with a silver-grey color on the upper side (Synge, 1974), inconspicuous bearing the staminate and pollinate flowers before the leaves. Flower buds are formed mostly on 2-year-old wood, differentiated during the previous growing season. In Sikkim Hills, the flowering starts in the month of March for a week to ten days and fruiting starts from April, ripens during late October-early November and retains till March to April. Seabuckthorn has found to possess vivipary (Basistha et al., 2001). Berries are green when immature and turn yellow when ripe in mid Sept to Oct. The berries lack an abscission layer and do not fall from the plant at maturity. The seabuckthorn plant grows in symbiosis with the nitrogen-fixing bacteria *Frankia* and therefore does not have any great demands for additional nitrogen (Singh et al., 2003). It develops an extensive root system rapidly and is therefore an ideal plant for preventing soil erosion and land reclamation. It was reported that the species can withstand temperatures from \(-43^\circ\) to 40°C (Lu, 1992). It is considered to be drought resistant (Heinze and Fiedler, 1981); however, irrigation is needed in regions receiving <400 mm of rainfall per year for better growth (Li and Schroeder, 1996).

### 1.3 Uses of Seabuckthorn

Seabuckthorns are reported to be used for centuries in Europe and Asia. Recently, researchers from around the world have paid considerable attention for nutritional and medicinal value of its fruits. The fruits are rich in carbohydrates, protein, organic acids, amino acids and vitamins. The concentration of vitamin C in seabuckthorn fruit, ranged from 100–300 mg/100 g fruit, is higher than strawberry,
kiwi, orange, tomato, carrot, and hawthorn (Bernath and Foldesi, 1992; Lu, 1992). Seabuckthorn fruits are also high in protein, especially globulins and albumins, and fatty acids such as linoleic and linolenic acids. Vitamin E content in seabuckthorn (202.9 mg/100 g fruit) is higher than wheat embryo, safflower, maize, and soybean.

Clinical tests on medicinal uses of seabuckthorn were first initiated in Russia during the 1950s (Gurevick, 1956). Seabuckthorn oil was formally listed in the Pharmacopoeia in 1977 and clinically tested in Russia and China (Xu, 1994). Mathews, 1994 reported the flavanoids, fatty acids and other bioactive compounds of *H. salicifolia* berries that might be capable of reducing the incidence of cancer. Seabuckthorn oil is attributed with most important pharmacological functions like anti-inflammatory, antimicrobial, pain killer, and promoter in tissue regeneration. It is also touted as a treatment for oral mucositis, rectum mucositis, vaginal mucositis, cervical erosion, radiation damage, burns, scalds, duodenal ulcers, gastric ulcers, chilblains, skin ulcers caused by malnutrition, and other skin damage. Different drugs are available in different forms, such as liquids, powders, plasters, films, pastes, pills, liniments, suppositories, and aerosols. Sea buckthorn oil extracted from seeds is popular in cosmetic preparations, such as facial cream (Li and Wang, 1998). Europe and Asia have numerous seabuckthorn products, such as tea from leaves, beverages and jam from fruits, fermented products from pulp, and animal feeds from leaves, pulp, and seed residues. The fruit juice is also used as cleaning agent for utensils and also in preparation of dye for coloring of clothes. The species is also considered as fine vegetation in improving soil fertility and restoring degraded sites in high hills (Gamble, 1972; Huxley, 1992).
1.4 Cultivation of Seabuckthorn

Seabuckthorn grows wild in nature in temperate areas of Europe and Asia (Yang & Kallio, 2006). The domestication and breeding process for seabuckthorn berries has been going on since the early 20th century and plants are now spread all over the world (Singh, 2003; Yang & Kallio, 2006). Recently, seabuckthorn has been recommended for orchard-type cultivation in British Columbia and the Prairie provinces. Seabuckthorn is transplanted or directly seeded in the spring and grows best in deep, well drained, sandy loam soil with ample organic matter. Water supplement is required in arid or semiarid areas. Soil acidity and alkalinity may ranges from pH 5 to 7. Seabuckthorn is sensitive to severe soil moisture deficits, especially in spring when plants are flowering and young fruits are beginning to develop (Li and McLoughlin, 1997). Seabuckthorn requires adequate soil nutrients for a high yield of good-quality fruit. It responds well to phosphorus fertilizer. Nitrogen fertilization delays the development of nodules after inoculation with Frankia. The recommended plant spacing for seabuckthorn is 1 m within the row and 4 m between rows to allow equipment access, with rows oriented in a north-south direction to provide maximum light. The ratio of male to female plants is important for maximizing the number of fruit-bearing trees. In British Columbia, with an orchard planting of 4000 trees/ha, a 1:6 to 1:8 male and female ratio is considered adequate. Pruning of seabuckthorn will increase the yield and lessen instability of fruiting, but due to the fruit setting on second-year branches, intensive cutting may result in a biennial yield. Various harvesting methods have been developed to replace hand-picking. One time-saving method reported is cutting of whole branches, which are immediately frozen to around -20 °C and the berries are thereafter shaken off when frozen. For promoting the growth of newly planted seedlings weed control is a
major task (Li and McLoughlin, 1997). Since early twentieth century, seabuckthorn breeding has been active in Russia, and some of that plant material (*Hippophae rhamnoides ssp mongolica*) was introduced to Sweden in 1986. China has 1.5 million hectares under seabuckthorn cultivation and 200 processing plants. Taking a cue from China, India today embarked on a major national initiative to grow seabuckthorn in one million hectare under the crop's ambit by 2020.

1.5 **Background and importance of the study on Seabuckthorn**

The analysis of nutrient and chemical composition, pharmacodynamics and toxicology of seabuckthorn by Chinese scientists resulted that seabuckthorn was a medicinal food containing many kinds of vitamins, trace elements, amino acids and other bioactive substances, such as β-carotene, VC, VB1, VB2, VK1, Zeaxanthin, lycopene, flavonoids, folic acid, sitosterol, triterpene, fatty acids, tannin acid, 5-HT (5-hydroxytryptamine) and umbelliferone, etc. In the former USSR it was discovered that the fruits of seabuckthorn contained more than 190 kinds of bioactive substances, and the oil contained 106 kinds of such substances. Of these, there were 6 kinds of fat-soluble vitamins, 22 kinds of fatty acids, 42 kinds of lipids and 36 kinds of flavonoids and phenols (Zhang, 1990). The medical products made from seabuckthorn include simple prescriptions as well as complex ones, e.g., oil solution, soft extract, membranous preparations and aerosols. Seabuckthorn oil can be used to treat burns, skin radiation lesions, cervical erosion, gastric and duodenal ulcer, etc. (Wu Fuheng, 1991). All these properties contribute a great extant to its use as a nutritional and medicinal food. Inspite of having many outstanding traits, the plant is still in an early stage of domestication. Increasing the seabuckthorn fruit yield is in progress and there
is also need to improve few more traits like thorniness, palatability and local climatic adaptation.

In India, mainly in the Department of Biotechnology (DBT), Government of India under Network programme on Hippophae (year 2009-2012), studies to explore the potential of this plant are taking place. Earlier, exploration and planting trials have been initiated especially in Himachal Pradesh, Leh, Ladhak and Sikkim (Singh et al., 1995; Basistha et al., 2001). However, the exploration and characterization of seabuckthorn germplasm in India is not yet completed. It is considered as important to make detailed taxonomic exploration, development of planting techniques, characterization of germplasm using morphological and molecular descriptors, and development of tissue culture protocol for large scale cultivation of this important plant species. The present work is a part of the DBT Network programme on Hippophae (year 2009-2012).

1.6 Objectives

Considering the importance of exploration, characterization and cultivation of seabuckthorn in India and particularly in North East India, which is a seabuckthorn grown area, a programme of study has been designed with the following objectives:

1. To study the distribution and ecology of seabuckthorn in Sikkim Himalayas and their characterization using morphological descriptors and molecular marker.

2. To study the phytochemical screening, antioxidant potential and antibacterial activities of the various parts of the plant.
3. To develop and optimize *in vitro* propagation systems using tissue culture technique for mass multiplication of selected clones and to study the clonal fidelity of the *in vitro* raised plantlets using molecular marker.