Chapter 1
Introduction

Sea buckthorn the general English term given to genus *Hippophae* was classified in 1753 in ***"Speciae Plantarum"*** by Karl von Linné at the position 1023 (Proorocu, 2009).

The genus belongs to the family Elaegnaceae, Order: Elaeagnales, Super order: Celastraneae, Subclass: Rosidae, Class: Magnoliopsida and Division: Magnoliophyta (Rajchal, 2009). It is a small family with five species viz. *Hippophae salicifolia* D. Don, *H. rhamnoides* Linn., *H. tibetana* Schlecht, *H. neurocarpa* S.W. Liu et T.N. He, and *Hippophae gyantsensis* Lian (Rousi, 1971). Among these, *H. rhamnoides* has further been sub-divided into eight sub-species (Rongsen, 1992; Schroeder and Yao, 1999; Bhattarai, 2003) (Table. 1.1). Other genera of Elaeagnaceae are *Elaeagnus* L. with 40 species and *Shepherdia* L. with only 3 species (Mabberley, 1998). Classification put forward by different authors from time to time for this genus may be referred at Table 1.2.

All the genera belonging to Elaeagnaceae have nitrogen-fixing bacteria in their root nodules (Rousi, 1971; Soltis *et al.,* 1995). The name *Hippophae* (Latin words ***Hippo*** means horse and ***Phaos*** means to shine) reflecting its uses in ancient Greece (Bhattari, 2003). In ancient Greece, the horses gained weight and attained shiny coat after people used *Hippophae* as fodder (Subedi and Adhikari, 2001).

The genus is locally termed as *Tarobo* (Bhutia), *Ree-singri* (Lepcha) and *Amilchi/ Tarechuk/ Ghanguru/ Achuk or Dalle chuk* (Nepali) in Sikkim (fig. 1.2 and 1.3). Siberian Pine apple, as referred by the Russians, is a fascinating, eco-friendly, cold Himalayan plant. It is native to Europe and Asia, has been known and used by humans for centuries (Rongsen, 1992; Dhakal, 2001).

Sea buckthorn (*Hippophae* L.*),* 2n=24 (Li and Schroeder, 1996) is mainly

Table 1.1: Species and sub-species of *Hippophae* L. as per recent taxonomical studies.

Genus: *Hippophae* Linn.
Family: Elaeagnaceae.
Species:

Group	Species	Sub-species
Coatless	*Hippophae salicifolia* D. Don	
	Hippophae rhamnoides Linn	*rhamnoides* Rousi
		Sinensis Rousi
		carpatica Rousi
		caucasica Rousi
		turkestanica Rousi
		mongolica Rousi
		yunnanensis Rousi
		fluviatilis Rousi
Coat	*Hippophae tibetana* Schlecht	
	Hippophae neurocarpa S.W. Liu et T.N. He	
	Hippophae gyantsensis (Rousi) Lian	

used for economic and ecological purposes. It is a dioecious, nitrogen fixing, actinorhizal, wind-pollinated plant (Jeppsson *et al.,* 1999; Arne Rousi, 1971), deciduous, thorny willow -like pioneering, shrubby or big tree up to 10 m in height. The genus has been reported to grow in low humid (15%), alluvial gravel, wet landslips, various soil conditions, hills, gully tops and riverside (fig. 1.1 and 1.2) with brown rusty-scaly shoots (Rongsen, 1992; Banjade, 1999; Basistha, *et al.*, 2009a). *Hippophae* species are fast growing (Rongsen, 1992), hardy woody plant often used in prairie conservation programmes (Schroeder, 1988), which is able to grow and survive well with low precipitations (300 mm), in soils with pH of 9.5 and 1.1% salts (Rongsen, 1990). The whole plant (fruits, roots, leaves and stem) is economically important (Fuhen *et al.*, 1999).

About 14 million hectare or more of natural sea buckthorn orchards are distributed widely over the world (Rongsen, 1992) ranging between 27°-69° N latitude and 7° W to 122° E longitude (Rousi, 1971; Pan *et al.*, 1989; Yu, *et al.*, 1989) spreading over 3500 km from east to west (Rongsen, 1990). Generally the global distribution pattern of sea buckthorn shows that the plant is concentrated mostly in the cold temperate regions of Hindu-khush Himalayas (Rongsen, 1990; Subedi, 2007), parts of Europe and former USSR as well as Scandinavian region (42 countries) (fig. 1.6). Sea buckthorn

Table 1.2: Overview of systematic treatment of *Hippophae* L. Taxa marked with an asterisk (*) were used in earlier classifications, but have never been validly published or transferred. (Source: Bartish *et al.*, 2002).

Servettaz (1908)	**Rousi (1971)**	**Avdeyev (1983)**	**Lian & Chen (1993)**	**Hyvonen (1996)**	**Lian et al., (1998)**
Hippophae rhamnoides	*Hippophae rhamnoides*	*Hippophae rhamnoides*	Sect. *Hippophae*	*Hippophae rhamnoides*	Sect. *Hippophae*
ssp.§ *rhamnoides*	ssp. *carpatica*	ssp. *rhamnoides*	*H. rhamnoides*	ssp. *carpatica*	*H. rhamnoides*
ssp. *Salicifolia*	ssp. *caucasica*	ssp. *salicifolia*	ssp. *carpatica*	ssp. *caucasica*	ssp. *carpatica*
ssp. *Tibetana*	ssp. *fluviatilis*		ssp. *caucasica*	ssp. *fluviatilis*	ssp. *caucasica*
	ssp. *gyantsensis*		ssp. *fluviatilis*	ssp. *gyantsensis*	ssp. *fluviatilis*
	ssp. *mongolica*		ssp. *gyantsensis*	ssp. *neurocarpa*	ssp. *mongolica*
	ssp. *rhamnoides*		ssp. *mongolica*	ssp. *mongolica*	ssp. *rhamnoides*
	ssp. *sinensis*		ssp. *turkestanica*	ssp. *rhamnoides*	ssp. *sinensis*
	ssp. *turkestanica*		*H. salicifolia*	ssp. *tibetana*	ssp. *turkestanica*
	ssp. *yunnanensis*		*H. sinensis*	ssp. *turkestanica*	ssp. *yunnanensis*
			ssp. *sinensis**	*H. salicifolia*	*H. salicifolia*
			ssp. *yunnanensis* *	ssp. *salicifolia*	Sect. *Gyantsensis*
			Sect. *Gyantsensis*	ssp. *sinensis*	*H. goniocarpa*
			*H. goniocarpa**	ssp. *yunnanensis*	ssp. *goniocarpa**
			H. gyantsensis		ssp. *litangensis**
			H. neurocarpa		*H. gyantsensis*
			H. tibetana		*H. neurocarpa*
					ssp. *neurocarpa*
					ssp. *stellatopilosa* *
					H. tibetana

§ssp=sub-species

plant can tolerate temperatures of –60°C and do not wither at a heat of 40°C (Rongsen, 1992). In India, three different species of *Hippophae* (viz. *H. rhamnoides* L., *H. salicifolia* D. Don and *H. tibetana* Schultz.) are naturally distributed in high altitude areas of Himachal Pradesh, (Lahul Spiti, parts of Chamba, Kinnaur, Kullu, Shimla and Kangra), Jammu & Kashmir (Leh and Ladhak) and parts of Uttar Pradesh (Singh *et al.,* 1995; Singh, 1998) and Sikkim (Basistha and Adhikari, 2003). In Sikkim Himalayas, the plant grows on the riverside, land slide areas, torrential slides and towards south-east facing aspects in Lachen and Lachung valleys of North Sikkim. *H. salicifolia* D. Don, in Sikkim, grows generally at altitudes ranging from 7844-10207ft.

Sea Buckthorn prefers colonizing in open habitats (Singh *et al.*, 1995). Among all *Hippophae* species, *H. salicifolia* succeeds fast and can grow in various soil conditions with low nutrients (Subedi, 2007).

Estimates have shown that sea buckthorn plantation can fix 45 kg of N_2 per hectare land, which is double the amount of N_2 fixed by Soya-bean plants (Rongsen, 1990). Sea buckthorn has life span of 60-350 years (Singh, 1998).

Sea buckthorn being one of the important crop, its propagation and agro-techniques had started way back in 1960 by the Russian scientist who collected and cultivated five varieties of the genera and perhaps was the first plantation in the world (Subedi and Adhikari, 2001). Presently, the largest plantation is located in Guskhrustalnyy, Vladimir State in the European part of former USSR (Rongsen, 1992). Being one of the important species for forestry and also in upgrading the sustenance of common people, sea buckthorn is being used in the most different ways and is considered as the plant of the 21st century.

Sea buckthorn is propagated using different means of agro-techniques like seed propagation, cuttings, layering in different soil conditions etc. (Auauzato and Megharini, 1986; Singh *et al.*, 1995; Basistha *et al.*, 2009b). The seeds retain their viability after indoor storage for three to four years. Under suitable conditions, they will germinate during any season of the year (source: Baker Dwight, www.winrock.org/forestry/factnet.htm).

In central Asia, one-two years old sea-buckthorn plants raised from seeds were used in complementary and comparative study of morphological, physiological and biochemical features, after harvesting. In 1940s sea buckthorn was planted for soil conservation, fuel, fodder and as wind break, apart from its medicinal and commercial importance (Rongsen, 1992). However, the level, quality and quantity could be substantially met through scientific plantations and managements. Demand for sea buckthorn fruit and oil by the industries has led to raise better quality of plants with high economic value. Therefore, propagation using cuttings and other vegetative means through selective plantations fulfils economic and ecological demands.

In the present study, different methods of propagation of *Hippophae salicifolia* were studied in controlled laboratory conditions, like germination performance of seeds in different soil based media, response to different concentration of growth regulators in seed germination, callus formation and rooting in layering, softwood and hardwood cuttings.

Variations in habitat of different sea buckthorn species bring variation in the chemical components (Wang, 1990). Sea buckthorn is rich source of medicinal property, nutrients and antioxidants. The fruits and seeds (fig. 1.3) are rich source of vitamins, especially vitamin C (300-1600 mg/100gm, which is 04-100 times more than any vegetable fruit), minerals, organic acid (2-4%, especially mallic acid which is higher than *Citrus* sp.1-2%), essential oils (unsaturated fatty acids around 86%), Palmitic acid (about 34%), oleic acid (about 32%) and palmitoleic acid (about 26%), etc. and possess a number of different medicinal properties like enhancing micro-circulation of bloods capillaries and nourishes skin/hair, combats cardiac problems, etc. They also have great potential for preparation of health foods and a variety of medicines (Centenaro *et al.*, 1977; Ansari, 2003; Zeb, 2004; Rongsen, 1992; Singh, 1998; Singh *et al.*, 2001) (table 1.4). It is considered to be a wonder plant known to be economically viable for the rural

Table 1.3: Composition of Vitamins content of sea buckthorn and other fruits and vegetables (mg/100g).

Items	Vit.A	Vit.B_1	Vit.B_2	Vit.C	Vit.K.
Orange	0.55	0.08	0.03	50.0	-
Sea buckthorn	11.00	0.04	0.56	300-1600	100-200
Tomato	0.31	0.03	0.05	8.0	-
Kiwi fruit	-	-	-	100-470	-

Source: *Occasional paper No. 20, 1992, ICIMOD, Kathmandu.*

Table 1.4: Food, Medicine and Cosmetics, Beauty cream made from sea buckthorn in Russia, South Asia and Tibet

Items	Products
Fruit juice	Syrup juice, Carbonated juice, Sweets, wine, Champagne Bears, Jam, Tea, Dye, Chocolates, etc.
Medicine	Cancer, Blood Pressure, Gastric Ulcer, Burns, Chemical danger, Plastic surgery, Cough, ageing, Memory loss, etc.
Beauty cream	Makes skin fair, Clean and delicate. Possess therapeutic efficacy on skin, wrinkle, Skin sclerosis, etc.
Cosmetics	Improves micro-circulation of capillary vessel, nourishes skin, hair and cares16 tropical diseases of scabies, etc.

Source: *Occasional paper No. 20, 1992, ICIMOD, Kathmandu*

population in many ways (Singh *et al.*, 1995). Table 1.3 shows the composition of vitamin content of sea buckthorn and other fruits and vegetables for reference.

The leaves too are rich in nutrients, antioxidants and bioactive substances (Rongsen, 2003; Small *et al.*, 2000). The young leaves contain high nutrient, carotenes and flavonoids. Generally, vitamin C content in leaves is higher than the fruit. It is also used as one of the most important raw materials for the extraction of vitamins and flavonoids. Sea buckthorn leaves also controls the growth of cancer cells in liver (Zhao *et al.*, 1999).

In case of Sikkim, fruit juices are traditionally used in dyes, making pickles, jam and treating some common ailments like cough and cold. The fruit pulps are also used to treat fever, diarrhea, scabies, constipation and other intestinal disorders. The matured sticks/branches of these trees are used for fencing work and making hedges around apple orchards and vegetable gardens. The plants are also used as fodder and firewood (fig. 1.5). Sea buckthorn is also a good source of firewood (Rongsen, 1990). It is apparent from the above discussions that *Hippophae* fruits, leaves and barks have potential in medicine, cosmetic health and food products. However, there has been no report on antioxidant and medicinal properties of *Hippophae salicifolia* growing in Lachen and whole of Sikkim. Therefore, the present study also aims to study the antioxidant property of fruit, leaves and barks of both male and female plants of this species.

Sea buckthorn is used as a measure species for soil conservation because of its well-developed tap root system (Nepal *et al.*, 2000; Rongsen, 2003). The genus has extensive sub-terranean

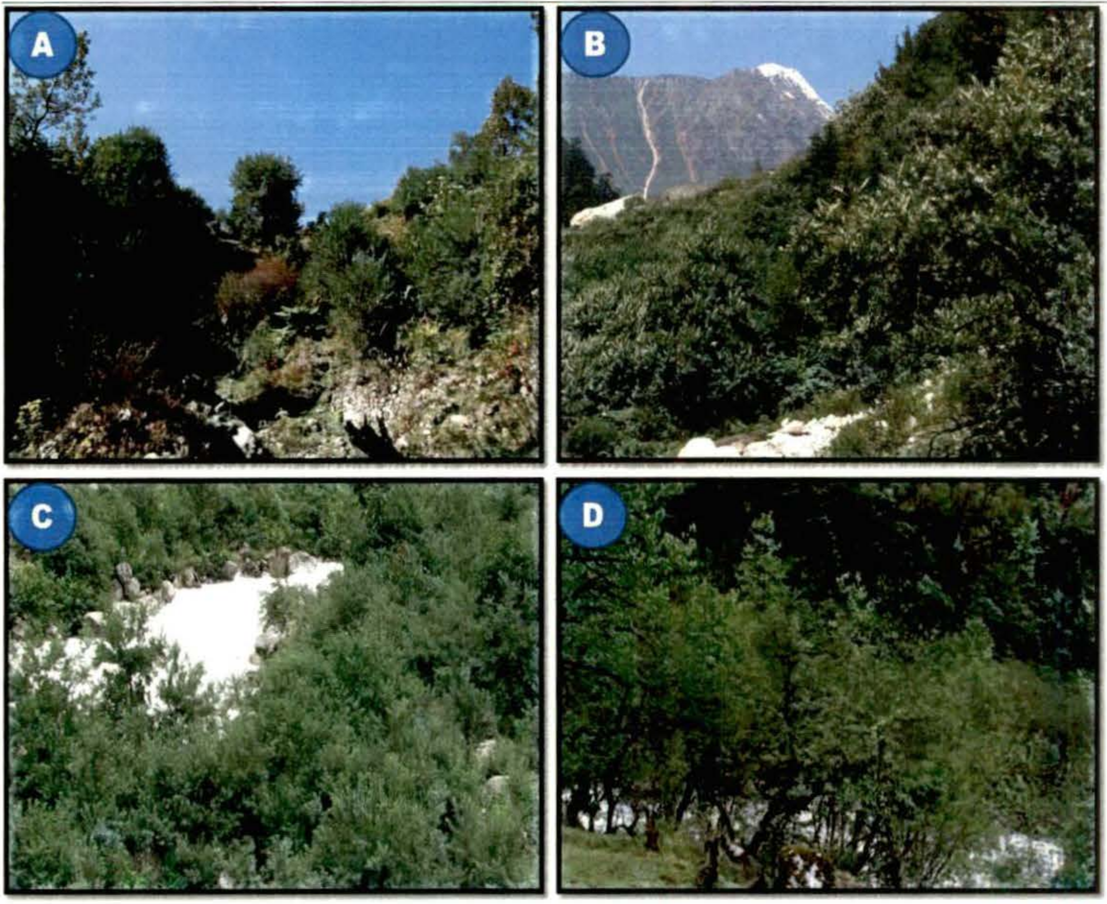

Figure 1.1: Natural habitat of *Hippophae salicifolia* D. Don. A & B: Non-riverine habitat and C & D: Riverine habitat

rooting system with strong soil binding ability, soil stabilization, riverbank control and water retention (Li, 1999) and in reclamation of marginal land (Bantle *et al.*, 1996; Schroeder, 1988; Li and Schroeder, 1999). Tap roots up to 3 m and horizontal roots up to 6-10 m have been observed (Jarsa, 1998). With its luxuriant foliage and strong root system, it can retain the soil from erosion.

Sea buckthorn also grows in areas with compressed soils (with less aeration and less water), and has symbiotic relationships with nitrogen fixing actinomycetes known as *Frankia* in its root nodules (fig. 1.4). Hence, it can be planted even in marginal soils (Akkermans *et al.*, 1983; Dobritsa and Novik, 1992; Rongsen, 1992).

Though people observed "bump" like structures in the root nodules of leguminous plants, nitrogen fixation was unknown to all until Hellriegel and Welfarth (1886) first announced in (Berlin) Germany that roots nodules of peas utilize atmospheric N_2 for their growth. This landmark discovery opened a new era for understanding science behind N_2 utilization by plants

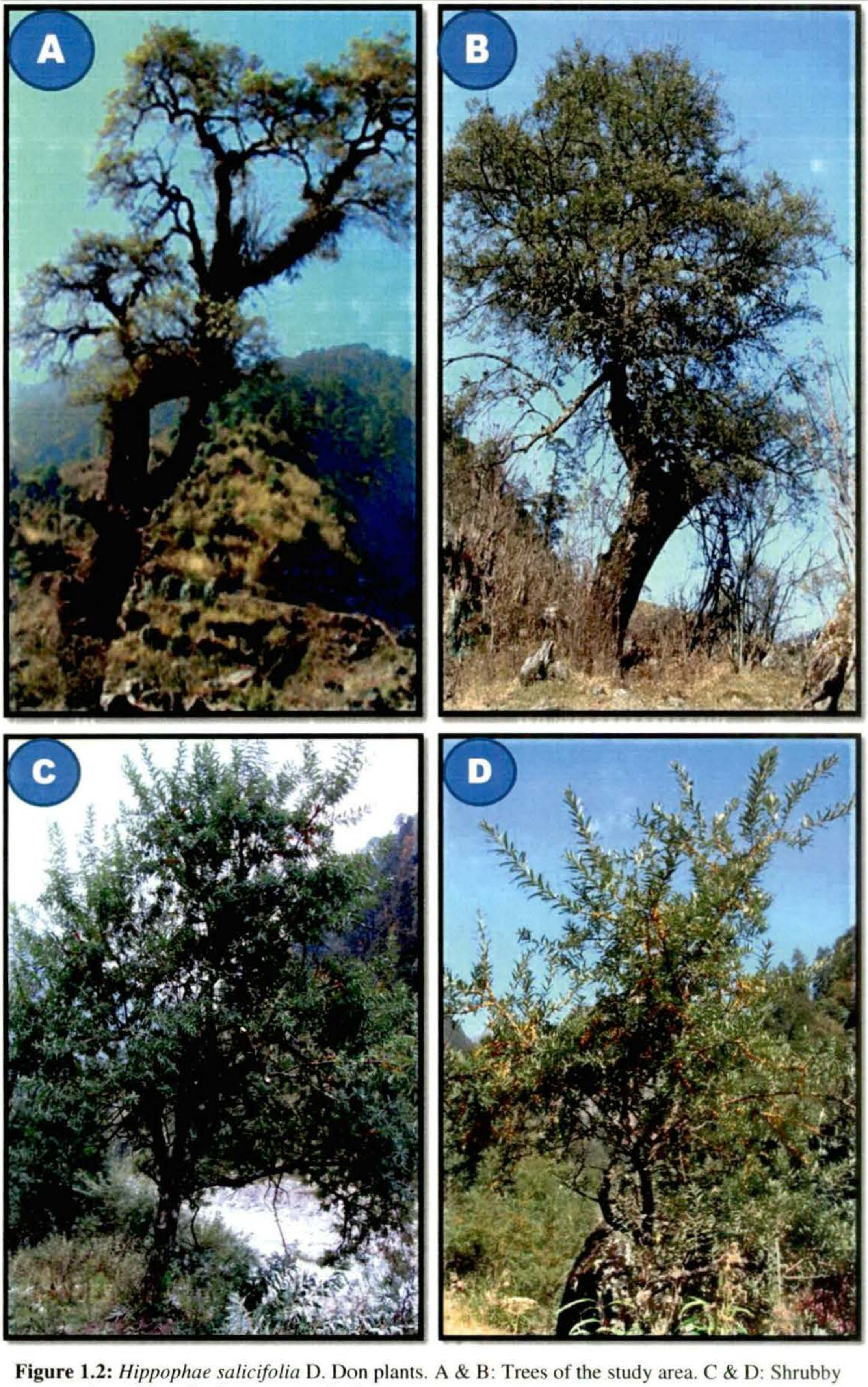

Figure 1.2: *Hippophae salicifolia* D. Don plants. A & B: Trees of the study area. C & D: Shrubby trees, male and female plants, respectively

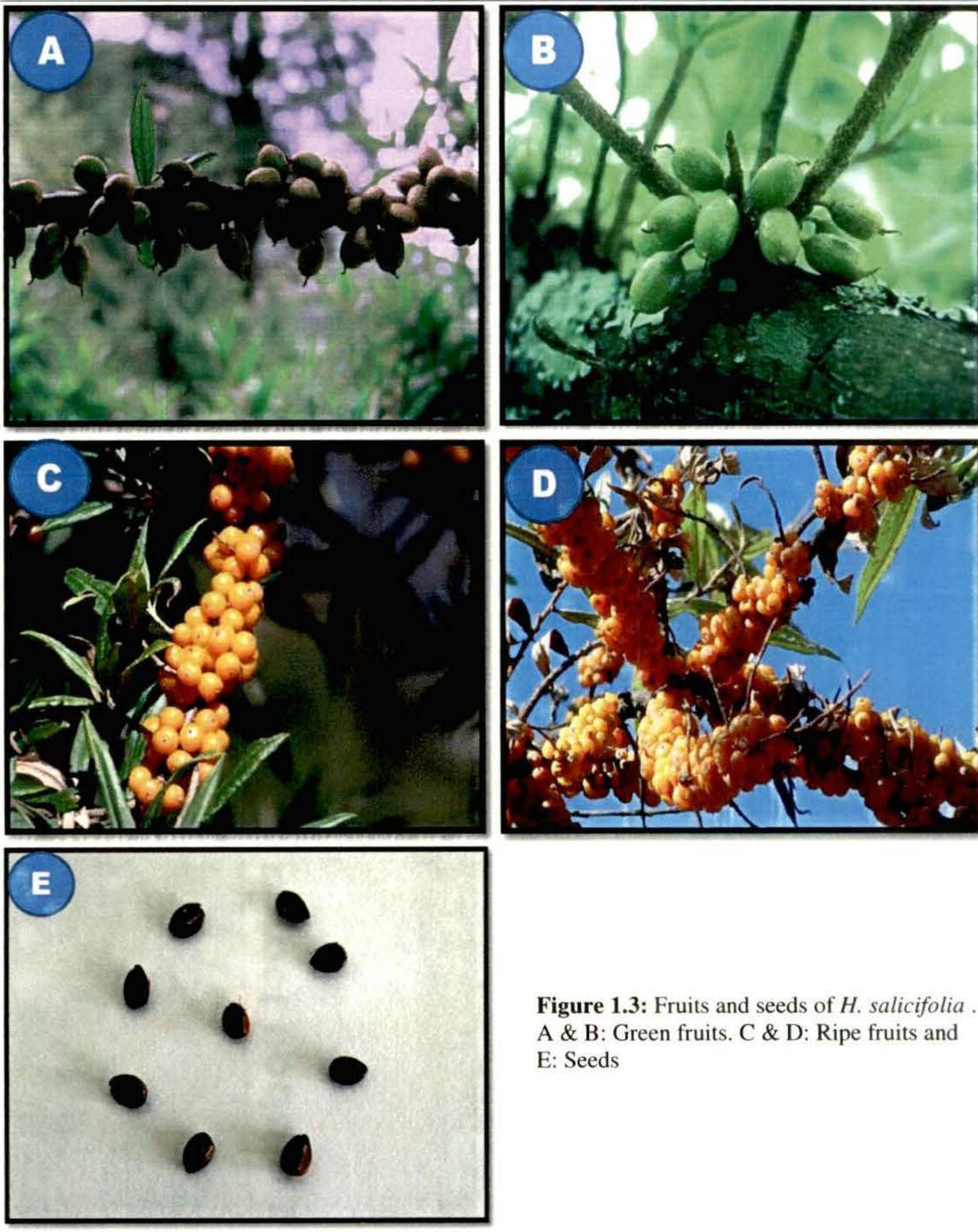

Figure 1.3: Fruits and seeds of *H. salicifolia* . A & B: Green fruits. C & D: Ripe fruits and E: Seeds

and microbes, apart from solving other confusions and controversies on sources of nitrogen for plants (Sen, 1996).

The complex mechanism of fixing of atmospheric nitrogen by the bacteria in legume root nodules and other microorganisms (both free living and symbiotic) has now been well explored by scientists for better understanding of this complex process of reducing nitrogen to ammonia with the aid of enzyme nitrogenase, and further assimilation to amino acids and other bio-molecules into biotic processes by various plants.

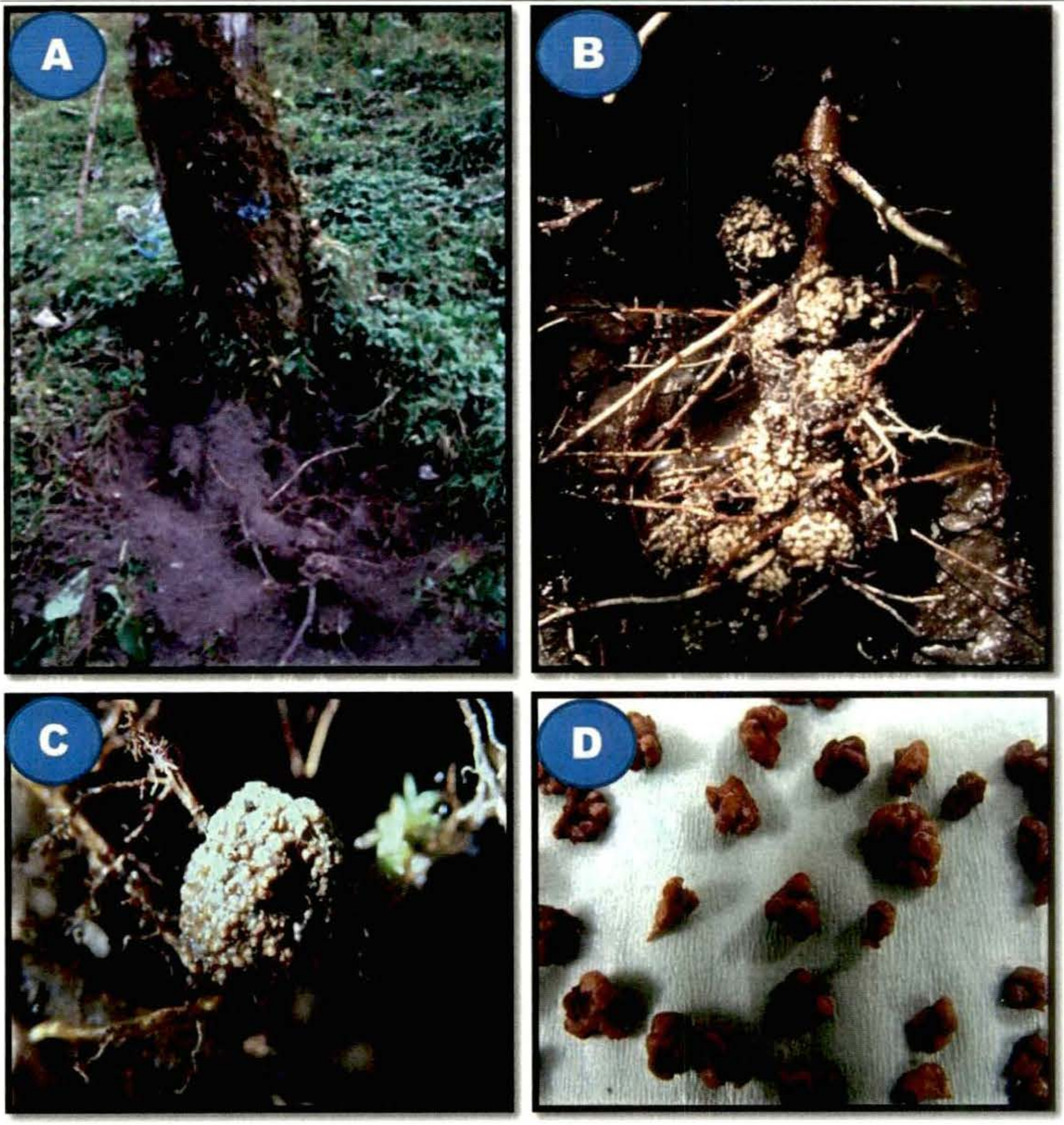

Figure1.4: Root nodule of *Hippophae salicifolia*. A: Nodule in natural habitat. B: Clump of nodules adhered to the roots. C: The individual nodule lobe cluster and D: Scattered nodular lobe

Almost all the nitrogen fixing microorganisms share two properties in common. One of course is the ability to fix nitrogen and the other is that they all are prokaryotes except *Eriophorum vaginatum*, artic sedge which prefers using organic nitrogen for its growth (Sen, 1996).

The nitrogen fixing organisms can be grouped into four groups'-rhizobia (legumes plant symbiotic bacteria), Cyanobacteria (blue green algae), *Frankia* (actinomycetes which associates with mostly woody flowering plants) and *Azospirillum* sp. (loosely associated and colonizes on the epidermis of host species like maize, wheat and rice) (Vande Broeak *et al.*,1993; Giller, 2001). Among all the symbiotic nitrogen fixer, the gram

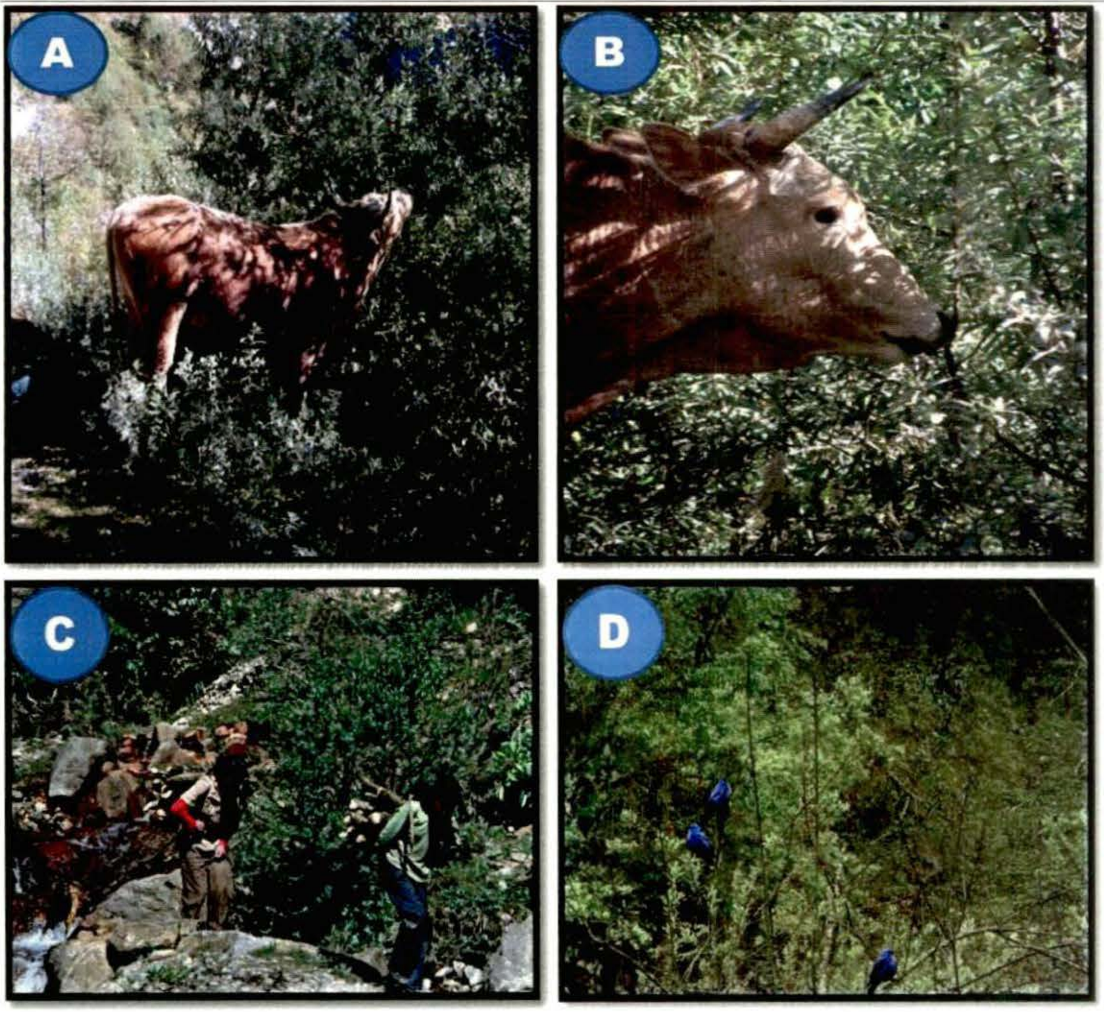

Figure 1.5: *H. salicifolia* as sustainable means for different components of temperate ecosystem

+ve actinomycetes of the genus *Frankia* (Goodfellow and Cross, 1984) are more significant because of their association with large number of woody dicotyledonous angiosperms' known as the actinorhizal (*actino* from actinomycete, and *rhiza* the plant root) plant (Tjepkema and Torrey 1979; Baker and Schwintzer, 1990), which includes *Hippophae salicifolia* too.

Actinorhizal plants can thrive in various types of climates and ecosystems like Arctic Tundra *(Dryas)*, forests (*Alnus, Casuarina, Shepherdia, Comptonia, Coriaria*), coastal areas and river banks (*Elaeagnus, Hippophae, Casuarina*), (Swensen, 1996), slopes and gullies (*Hippophae, Alnus*) either lone stands or mixed (*Alnus, Hippophae*). Except *Datisca*, which is herbaceous, other actinorhizal plants are perennial dicotyledonous, woody shrubs or trees.

Eastern Himalayas region of India has high biological diversity of angiosperms. About 70% of angiosperms have been documented from this region. It is also a region of high diversity of actinorhizal plants (Pradhan, 1993).

Actinorhizal plants are pioneering species in the nitrogen-poor and disturbed soils. They have been widely used in afforestation programmes and agro-forestry ecosystems (Ruskin, 1984; Seiler and Johnson, 1984), land reclamation, biomass production and forest restoration (Benson, 1982), timber, fuel, etc.

Actinorhizal plants have gained importance globally in the recent past because of their potential in reforestation of anthropically degraded areas like mines, spoils, dam dykes, clear-cut forests or over-exploited agricultural and producing timber, windbreak, fuels, fodder, ornaments (Dawson, 1990; Diem and Dommergues, 1990; Kucho *et al.*, 2010). The amount of nitrogen fixed by most of these plants is almost the same to that of leguminous plants (40-350 Kg N/ha/year) (Torrey, 1978) and few

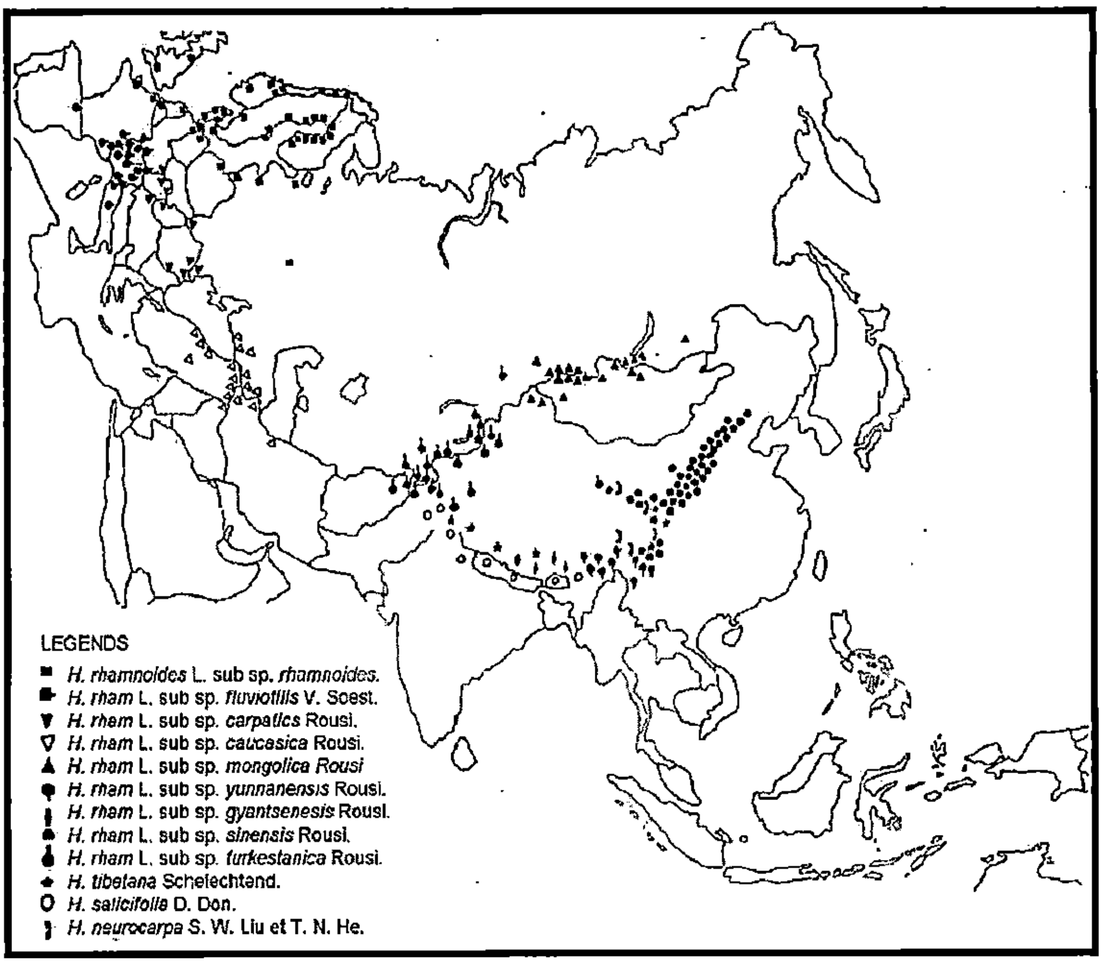

Figure 1.6: Distribution of sea buckthorn (*Hippophae* L.) in Europe and Asia [Source: Arne Rousi (1971); Lu Rongsen (1990)].

species can even release up to 70% of fixed N_2 to the ecosystem (Schwintzer, 1984).

Rhizobium strains and *Frankia* strains have marked differences in gram staining (Diouf *et al.*, 2003). There is a marked difference in the nodules produced by *Frankia* in actinorhizal plants and the *Rhizobium* strains in leguminous plants. This is because there is extreme diversity of the host plants and the symbionts (Silvester *et al.*, 1990). The infection site in the leguminous roots are usually within endodermis and inner cortical cells covered by vascular tissues, whereas in actinorhizal roots, nodules normally have a central stele that has infected tissue adjacent to or around it.

Actinorhizal root nodules endophyte could establish their identity only in the year 1964, after its prokaryotic structure in *Alnus glutinosa* and *Myrica cerifera* was revealed (Sen, 1996). After several experiments for many years, report on first strain of *Frankia*, CpI1 (now known as HFPCcI1) was reported in 1978 by Torrey and his co-workers from *Comptonia peregrinn* (Callaham, *et al.*, 1978) and the ability of the organism to re-infect the host plant and re-establish the symbiosis was confirmed (Lalonde, 1978). This report demonstrated that *Frankia* strains constituted a previously not described group of soil actinomycetes that are facultative symbiotic with higher plants. This association among the plant root and the endophyte was termed as 'actinorhiza' by analogy to mycorrhizal associations (Tjepkema and Torrey, 1979).

The term *Frankia* has been in use since long time when it was first coined in 1886 by J. Brunchorst to honour his mentor A. B. Frank (Quispel, 1990). The groups of microorganisms that induce root nodules, capable of fixing atmospheric N_2 in non-leguminous plants are broadly classified under the genus *Frankia*. *Frankia* are filamentous gram-positive bacteria of the family Actinomycetales. This ecological significance has made this microorganism one of the important subjects for the researchers (Baker and Schwintzer, 1990).

Successful isolation of *Frankia* from the root nodules have been made from almost all actinorhizal plants except *Ceuthostoma, Kentrothamnus, Chamaebatia, Dryas* (Benson & Silvester, 1993) and *Adolphia*. (Huss-Danell, 1997). Till date strains from 25 genera of actinorhizal plants have been isolated all over out of which only nine genera showed to give effective nodules when plants of their hosts were re-inoculated. This may be due to non-infectiveness of *Frankia* on their genus host or nodulations were not examined.

As a consequence of many research works, *Frankia* is now relatively well defined. Three cell types are produced by *Frankia,* which are useful in studying prokaryotic behavior of the organisms. New species have been proposed, physiology and growth characteristics of some species have been studied in details and step towards molecular and genetic studies are going on. Study of genetic diversity at the molecular level, of the available strains, whose infectiveness and effectiveness are yet to be understood, has made these organisms more interesting for the studies of host-symbiont relationship and their interactions at different phases of N_2 fixation.

Under *in vitro* conditions, *Frankia* are characterized by septate and tightly interwoven hyphae, sporangia and vesicles. The sporangia are multilocular at terminal or intercalary regions. Vesicles, the site of nitrogenase activity are developed only when the culture media is either devoid of nitrogen or less nitrogen (Tjepkema, *et al.*, 1980; Zhang and Benson, 1992). They are encapsulated with lipids, spherical shaped measuring 2-6 μm in diameter and laterally or terminally attached to hyphae by a short encapsulated stalk (Sen, 1996). In natural condition, *Frankia* morphology may be modified due to variation in presence and absence sporangia, their size, shape and presence of vesicles. This morphological change is influenced by the host plant during symbiotic process (Benson and Silvester, 1993).

The rhizospheric microflora has become one of the important economic factors for tropical agro-forestry. Their use in the improvement of plant productivity with decrease in the use of chemicals in the form of insecticides and pesticides has started showing good results (Okon and Hader, 1987). The richness of *Frankia* diversity in the natural forest is governed by the richness of the actinorhizal species along with ecological and geographical factors like slopes, aspect, precipitation, etc.(Chen *et al.,* 2008).

After extensive review of *Frankia* by Newcomb and Wood in 1987, there has been significant development in understanding the microorganism, particularly its vesicle structure. The anatomy of root nodules reveal various shapes of vesicle from elongated to pear shaped or globose (Torrey, 1985). Cell component of *Frankia* are delicate in nature, particularly the extra-cellular covering of vesicle, which has caused some difficulty in structure analysis through electron microscopy. But techniques like freeze fracture and freeze substitution has eased the procedure of electron microscopy still with some problems in chemically fixed specimens (Newcomb and Wood, 1987).

Ecological studies and identification of *Frankia* in nodules or soil has been difficult due to lack of reliable and convenient procedures, but development of molecular studies like Polymerase Chain Reaction (PCR), sequencing techniques and DNA hybridization in the recent past has made the whole task easier and convenient for studying *Frankia* population and phylogeny.

Presently, *Frankia* analysis from soil (Myrold *et al.*, 1990), culture isolates (Simonet *et al.*, 1991; Bosco *et al.*, 1992; Normand *et al.*, 1992) and nodules (Simonet *et al.*, 1990; Nazaret *et al.*, 1991) can be done through molecular approaches. *Frankia* can directly be isolated from uncultured root nodules and cultures always may not be required for these studies. Development of PCR techniques using *Frankia* specific primers to 16S rRNA genes, 16S-23S rRNA intergenic region, *nif*D-*nif*K intergenic region genes, etc. have been mostly applied in all cases of *Frankia* isolates. (Benson *et al.*, 1996; Clawson *et al.*, 1998; Jamann *et al.*, 1993; Jeong *et al.*, 1999; Murry *et al.*, 1997; Nalin *et al.* 1995; Nazaret *et al.*, 1991; Normand *et al.*,1996). It has become easy for the systematic, evolutionary, diversity and ecological study of different *Frankia* species by sequencing the PCR amplified genes of these microorganisms (Olsen *et al.*, 1986; Woese, 1987). From different publications and references, it has been revealed that molecular approaches in the study of *Frankia* isolates both from culture (*in-vitro*) or nodules have been useful in explaining phylogenetic relationship within the genus or species. (Giovannoni *et al.*, 1990; Mirza *et al.*, 1992; Nazaret *et al.*, 1991; Nick *et al.*, 1992). This technique is at par obvious for defined molecular phylogeny groupings of *Frankia* (Clawson and Benson 1999; Mirza *et al.*, 1994a; Rouvier *et al.*, 1996).

Specific primers and probes have been

developed due to increase in *Frankia* sequence database for PCR and in situ hybridization. Now amplification of specific segment of *Frankia* is also available (Mishra *et al.*, 1991). Due to these sophisticated process and techniques, ecological influences on introduced or indigenous *Frankia* populations are being studied by different researchers all over the world.

From the previous work on *Frankia* (Reddell and Bowen, 1985; Sougoufara *et al.*, 1992), it is revealed that host has a significant role to play compared to the microsymbiont during symbiosis but later experiments through physiological data indicates that there is a direct involvement of the host plant, but lacks molecular evidences (Bajwa, 2004). However, some ecological, agrotechniques and antioxidant studies on the host plant too were taken up in this study during the study of genetic diversity of *Frankia* to correlate with its host and the ecological niche. However, it was felt that some more studies in this line were necessary.

Frankia research is an ongoing process of many workers since long time. There may be procedural hurdles but procedures like molecular techniques may answer many unsolved questions in *Frankia* research. Using proven techniques plays a vital role in solving some issues. Study of genetic diversity of *Frankia* in its natural population is one of the interesting study using molecular tools. The present experiments and investigations were taken up with the following objectives:

•Environmental studies of native *Hippophae* of Lachen Region (field based study).

•Soil analysis for micro-nutrient studies of *Hippophae* growing area.

•Isolation and Characterization of *Frankia* associates with roots of native *Hippophae*.

•Isolation of genomic DNA.

•Study of genetic diversity of *Frankia* through PCR and RFLP.