CHAPTER 14

General discussion

Medicinal plants are one of the important groups of bio-resources, which are the base line for the life of human beings as well as for other animals. Most of the medicinal plants are found in the wild habitat and they help in the progress of human civilization in different aspects, i.e. pharmaceutical, economical, commercial, social etc. Due to their habitat loss, the population of this group of plants is gradually decreasing. The main cause of habitat loss is due to the anthropogenic activities. Due to degradation of their habitat and decline of population, perhaps this important of group plants will be gone forever from the nature as well as some of them have already become endangered. FAO (2003) reported that due to the continuous exploitation and substantial loss of their habitat during the past 15 years, the population of several medicinal plant species has declined from the wild over the years. The only solution to protect them is sustainable use with proper utilization of these bio-resources. Keeping the above in mind, we should adopt some conservation measures including both ex situ and in situ for their sustainable use for the benefit of the future generation.

In the present study four medicinal as well as endangered plant species were taken into consideration, i.e. Homalomena aromatica (Roxb.) Schott., Smilax glabra Roxb., Bulbophyllum careyanum (Hook.) Spreng. and Paphiopedilum spicerianum (Rchb.f.) Pfitz. Among these four plants, Homalomena aromatica (Roxb.) Schott. is an aromatic plant and Bulbophyllum careyanum (Hook.) Spreng. and Paphiopedilum spicerianum (Rchb.f.) Pfitz. are two important orchid species of Southern Assam. The fourth plant species Smilax glabra Roxb. is a woody climber. First three plant species are considered as medicinal plant for their use into curing of different ailments and Paphiopedilum spicerianum (Rchb.f.) Pfitz. is used as a highly demanded cut flowers due to their unique beauty of the flowers and also for their aesthetic value. On the other hand the target plant species are considered and identified under the different categories of threatened plants (RET plants).

Vegetative propagation is one of the potential and useful methods of asexual reproduction of those plant species, which are economically important but their population is very less
in the natural habitat as well as they are difficult to propagate through seeds (Banday et al., 2014). This method is easy and effective for the mass multiplication and production of true-to-type plants and also it preserves the genetic characters (Butola and Badola, 2006; Banday et al., 2014). This method of propagation is also considered as ideal for rapid multiplication of a species under threat, while trying to maintain certain desired characteristics (Hartmann et al., 2002; Tchoundjeu et al., 2004). In the present study, both in vivo and in vitro vegetative propagation were taken into consideration. The target plant species, i.e. Homalomena aromatica (Roxb.) Schott., Smilax glabra Roxb., and Bulbophyllum careyanum (Hook.) Spreng were successfully propagated vegetatively under the greenhouse and nursery in pot culture (in vivo conditions) under varied conditions. Mainly the rhizome of the experimental plant species were used as an experimental planting material. From the results, it was observed that these plant species could be propagated from the rhizome. The young and mature stem of Smilax glabra Roxb. were also taken as a planting material for vegetative propagation, but there was no growth response. Therefore only rhizome was taken into consideration. While on the other hand, in the in vitro condition, the three experimental plant species were successfully propagated by micropropagation technique.

Southern Assam is one of the important home lands for different types of medicinal as well as endangered plants. The ethnic communities of this region use the different parts of the medicinal plants in their traditional health care system. But due to the lack of proper utilization and awareness among them, these plants are over exploited. Homalomena aromatica (Roxb.) Schott. and Smilax glabra Roxb. are two of the such important medicinal as well aromatic plants of this region. The fresh and dry rhizomes of Homalomena aromatica (Roxb.) Schott. are used as a raw material by the different industries. About 400 MT of dry rhizomes is exported from Southern Assam to different places of India. But the wild population of these species is declining gradually due to the illegal extraction and over exploitation of its rhizome. In the present work, an attempt was made to establish the production of individuals by vegetative propagation as well micropropagation of some plant species (i.e. Smilax glabra Roxb., Homalomena
Rhizome is one of the important vegetative parts of the rhizome producing plants. It is the swollen and compressed underground stem with short scaly leaves and vegetative buds. A piece of rhizome with a bud, if planted, will grow into an independent plant (Somashhekhar and Sharma, 2002). The rhizome is used as a propagating material in the vegetative propagation of some important plant species. In the present work, rhizomes of *Homalomena aromatica* (Roxb.) Schott. and *Smilax glabra* Roxb. were used as a planting material in the different experiment. The rhizomes were cut into transverse sections with one active bud, which helped in the vegetative propagation of the target plant species. Rawat *et al.*, (1992) reported that the transversely segmented tuber or rhizomes have the potential to regenerate into new plantlets with well-differentiated root and shoot in the vegetative propagation. The three different cuttings of the rhizomes of *Homalomena aromatica* (Roxb.) Schott. i.e. Cutting 1 (C1, upper portion), Cutting 2 (C2, middle portion) and the Cutting 3 (C3, lower portion) have shown good potential source of planting material for vegetative propagation of the said species. Kuniyal (1999) reported that the apical segments of the rhizome produced single shoot, while sub-apical, middle and basal portions were also able to regenerate several sprouts. The cutting 2 (C2), i.e. in the present experiment, the middle portion of rhizome has shown to be the best propagating unit.

Orchid is considered as a highly evolved and unique group of flowering plants with specialized adaptive excellence (Hedge, 2012). This group is also identified to be extremely endangered group of plants around the entire globe (Jezek, 2003). According to the IUCN Action plan (1999), orchids are identified as amongst the world’s most diverse and widely distributed plants (Sibin *et al.*, 2014). North East India is one of the important habitats for the orchids; it contributes ± 839 species under 144 genera (Hedge, 1997, 2000, 2001; Kataki *et al.*, 1984. Manilal and Sathish Kumar, 2004; Pradhan, 1976, 1979). However, now a days, the population of different orchid species are declining due to over exploitation, habitat destruction and deforestation. All the orchid species are protected under Wild Life (Protection) Act, 1972, and treated as Protected species under
CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) ((Hedge, 2012). Vegetative propagation is one of the important methods for the conservation of different orchid species. By this method, a true to type progeny or offsprings are produced from the mother plant. The advantage of the vegetative propagation of orchids is relatively high speed as the new plants are produced by the traditional way (Jezek, 2003). There are different types of methods of vegetative propagation, which are used for the propagation of orchid species.

In the present work, an epiphytic orchid *Bulbophyllum careyanum* (Hook.) Spreng. was considered for the vegetative propagation *in vivo* under green house condition. Three different growing conditions i.e. 1) Growing Condition Number 1: Brick + charcoal + wood ( 1:2:1) with soil in pot culture, 2) Growing Condition Number 2: Brick + coconut husk + charcoal ( 1:2:1) with leaf mould in pot culture, 3) Growing Condition Number 3: Trunk of tree *Bauhinia variegata* L. were used for the vegetative propagation of this orchid species. In this experiment, the method of division of the orchid was applied. Although it is an epiphytic orchid, but it also can grow in pot culture with appropriate potting mixture under green house condition. From the result it was observed that the above mentioned three growing conditions are suitable for the vegetative propagation of this orchid species, but the growth parameters were found to be varied in different growing condition. Jezek (2003) reported that the main conditions for the survival of epiphytic orchids are certain levels of humidity, temperature, light and nutrition. Water supply plays an important role for the morphology and survival of the epiphytic orchids under control or artificial condition. According to Jezek (2003) the morphological structure and survival of the epiphytic orchids are adopted to both regular and irregular water supplements during the day as well as during the course of the whole season.

Environmental factors are one of the important parameters for the vegetative propagation. Different environmental factors affect the vegetative growth and propagation of the plants. The rate of vegetative propagation and growth are found to be different during different seasons. Because different microclimatic conditions vary from time to time and they affect the vegetative growth directly and indirectly. In the present study, the two plant species, *Homalomena aromatica* (Roxb.) Schott. and *Smilax glabra* Roxb. were
attempted to propagate during the four different seasons (i.e. spring, summer, autumn and winter). But both the plant species could not be propagated during the winter season. Spring season is found to be favorable time for the vegetative propagation of these two plant species. Zheng and Feng (2006) reported that during the winter season, the extreme water stress condition from the outside atmosphere or due to fog or frost condition, reduces the plant growth, although plants can absorb some fog water directly through leaf and recover to the water balance.

Medium or substrate is one of the important factors in the vegetative propagation. Because the vegetative growth and development as well as rooting of the planting material always depends upon the types of substratum or the medium used. According to Bunt (1988) the quality of potting medium plays an important role for the successful growth of the plants in the containers under nursery conditions. On the other hand some species tend to root better in certain substrate (Butola and Badola, 2006). For better rooting, the substrate requirements for rooting are generally associated with their hydromorphic or xeromorphic status (Loach, 1985). Suitable growing medium is used to achieve improved rooting quality and subsequent plant growth during the vegetative propagation. (Thetford et al., 2001; Mialoundama et al., 2002). The medium should be free of disease causing pathogens, weeds, pests, nematodes and should have good water holding capacity and good drainage (Dole and Wilkins, 2005). Three types of natural substrate or medium i.e. soil, sand and leaf mould, were used in the present work. It was observed that the three natural substrate, i.e. soil, sand and leaf mould have affected the vegetative propagation of Homalomena aromatica (Roxb.) Schott. and Smilax glabra Roxb. both positively and negatively. The percentage of propagation and survival percentage of Homalomena aromatica (Roxb.) Schott. were found to be 100%. The three natural substrata were favourable for the vegetative propagation of this species. While soil was found to be the best medium for the vegetative propagation of Smilax glabra Roxb. The other two substrate sand and leaf mould were found to be significantly poor in the vegetative propagation of Smilax glabra Roxb. The rate of vegetative propagation was found to be varied i.e. 100% in soil, 40% in sand and 20% in the leaf mould, respectively.
Organic amendments always supply the nutrients to soil and improve the soil fertility. The use of organic fertilizers can increase soil fertility and enhance vegetative growth of the plants without leaving any harmful residue in the soil as well as in the harvested rhizome, which improves quality and yield. Lohani et al., (2011, 2012) reported that the application of FYM in the cultivated field is the traditional practice in Kumaun Himalaya for the better yield and crop production. The advantages of the application of FYM are reported by several workers in different times. Application of FYM always increased the biomass in different crop plants (Patidar and Mali, 2001; Saharan et al., 2001; Kasera and Sharan, 2002). Lohani et al. (2012) reported that the vermicompost increased moisture content of the soil and retained it quite some time. Application of vermicompost always improved the physical, chemical and microbial properties of the soil and its productivity. Increased soil fertility enhanced the vegetative growth of the plants and additional nutrition got stored in the underground rhizome. Several studies have also shown that the application of organic fertilizers reduces the incidence of soil borne diseases and pathogens (Faqir et al., 1995; Vanlauwe et al., 1996; Sarathchandra et al., 2001; Graham and Haynes, 2005). Atiyeh et al., (2000) have reported that vermicompost consistently promote biological activity which can cause plants to germinate, flower, grow and yield better than in commercial container media, independent of nutrient availability. It also contains large amounts of Humic substances (Tomati et al., 1987) and some of the effects of these substances on plant growth have been shown to very similar to the effects of soil applied plant growth regulators or hormones (Muscolo et al., 1999). In the present work, two types of organic amendments, (i.e. cow dung and vermicompost) were used in different dosages, (i.e. 100 gm, 250 gm and 500 gm per 2 kg of soil) for the vegetative propagation of the targeted two plant species (i.e. Homalomena aromatica (Roxb.) Schott. and Smilax glabra Roxb.). Due to the application of these two organic amendments, it was observed that the growth of the vegetative propagation of the target species was better than the control, i.e. without the application of organic amendments. Plant growth regulators play an important role in the rooting of the plants. They have been widely used in the vegetative propagation to improve rooting as well as the subsequent growth of the cuttings (Nadeem et al., 2000; Butola and Badola, 2007). Auxins are one of the important well-known rooting agents (plant growth regulator) and
their application (naturally occurring or synthetic) for large-scale multiplication of plants has been well documented (Hartmann et al., 2002). Nadeem et al., (2000), and Rawat et al., (1992) mentioned that IBA or NAA treatment has been found to be effective for the rooting of rhizome/tuber segments of *Podophyllum hexandrum* and *Aconitum atrox*. In the present work three types of plant growth substances (plant growth regulators) were used in the vegetative propagation of *Homalomena aromatica* (Roxb.) Schott. and *Smilax glabra* Roxb. IBA, IAA, GA₃ were used at different concentrations, i.e. 10, 20, 30, 50, 70, 90, 100, 250, 500 ppm. However, there was no positive response in the vegetative propagation of the targeted two above mentioned plant species. Only it was observed that the rhizomes got rotten due to the growth substances treatments as compared to control, i.e. without the application of growth substances.

Cultivation is one of the best conservation measures for the endangered medicinal plants, which helps in the increases their natural stock in the wild habitat. But it must be done by appropriate scientific way. It also helps to stop over exploitation and illegal extraction. Sher et al., (2010) suggested that the cultivation of medicinal plants is the only solution for the rapid conservation. Medicinal plants are one of the economically important renewable bio-resource, which provide the different raw materials to different industries of pharmaceuticals, perfumery, flavor, soaps and cosmetics. Now a days a numbers of agencies have recommended that the wild species of the medicinal plants may be brought under cultivation, because of the increasing growth in human needs, numbers of commercial trade as well as reduced natural stock (Lambert et al., 1997; Schippmann et al., 2002). Anon (2002) reported that the medicinal plant production through cultivation can reduce the illegal extraction and also may lead to reduction of environmental degradation and loss of genetic diversity as well loss of incentives to conserve wild population. *Homalomena aromatica* (Roxb.) Schott. is a highly demanded aromatic as well as medicinal plant of Southern Assam. But the wild stock, i.e. population of this species has declined over the year due to the anthropogenic pressure for the economic as well as commercial exploitation. On the other hand the plant is identified as endangered plant by the Ministry of Environment and Forest, Government of India (Anon 2012). Therefore an attempt was made to develop an agrotechnique for the conservation of this species under the agroclimatic condition of Southern Assam under field condition. In the
present work, the rhizomes have been used as a planting material with the treatment of above mentioned organic amendments, i.e. cow dung and vermicompost. Three types of cutting of rhizomes, i.e. C1 (upper portion), C2 (middle portion), C3 (lower portion) were successfully propagated with the treatment of the two organic amendments. Earlier report suggested that the 45× 30 cm² is the best spacing for cultivation of this species (Khan et al. 2012). But from the present work it can suggested that it can also cultivated at 45× 45 cm² spacing under the agroclimatic condition of Southern Assam. There is no cultivation practice in this geographical region as yet. A tremendous scope is available for the commercial and economic growth of this economically important plant species, which will help the progress of the life style of the people of this region, who are involved in the trade of this plant species.

Micropropagation is one of the important conservation initiatives which help to increase the population of a particular plant species within a particular period of time. This technique produces a large number of genetically identical plants within the realm of reality (Kaur and Pathok, 2014). This in vitro technique for rapid and mass propagation offers possibilities for ‘recovery’ of the endangered species, thus reducing the risk of extinction (Nadeem et al., 2000). According to Srivastava et al. (2010), micropropagation provides the best tool for large scale production of propagules, especially in case of endangered medicinal plants. This biotechnological tool offers a tremendous potential for the propagation of endangered and superior genotypes of medicinal plants, which could be released to their natural habitat or cultivated on a large scale for the pharmaceutical industry. Micropropagation or in vitro propagation of RET plants is generally undertaken to enhance the biomass and to conserve the germplasm, especially when their population is very less in the wild habitat. This is a viable alternative for the species, which are difficult to regenerate by conventional methods, i.e. vegetative propagation, seed germination; where population have decreased due to over exploitation by destructive harvesting. Now a days this biotechnological technique is widely used for the conservation of medicinal as well as the endangered plants. Micropropagation is an advanced propagation method for germplasm conservation to ensure the survival of the endangered plant species, rapid mass propagation for large scale revegetation and for genetic manipulation (Yadav, 2012). In the present study one endangered medicinal
plant, i.e. *Smilax glabra* Roxb. and two endangered orchid species i.e. *Bulbophyllum careyanum* (Hook.) Spreng. and *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. were successfully propagated by micropropagation. *Smilax glabra* Roxb. is a highly demanded endangered medicinal plant of Southern Assam. In this plant, seed germination is very less. Therefore an attempt was made to establish a protocol for the micropropagation of this species. Different parts of the plant body were used as explant source in the present work. Interestingly only nodal explant showed good response in the MS and MS modified media. But MS modified medium showed negative response in case of rooting. A successful micropropagation protocol was developed. Naturally orchids are propagated vegetatively by different ways. The seed germination of this plant group is rare due to lack of endosperm. Therefore seed germination of orchid species is only done with the association of mycorriza in nature. The two target orchid species, i.e. *Bulbophyllum careyanum* (Hook.) Spreng. and *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. were successfully propagated under *in vitro* technique. *Bulbophyllum careyanum* (Hook.) Spreng. is highly medicinal orchid species of southern Assam, while *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. is an endangered orchid species under CITES. At present the population of this two orchid species is very less. Although vegetative propagation is one of the conservation measures taken but it requires a large number of the plant parts or plant body. Therefore, micropropagation is the best and the suitable conservation measure for the orchid conservation. Keeping this point in view, this two important orchid species were successfully propagated by the micropropagation technique. The seeds of *Bulbophyllum careyanum* (Hook.) Spreng. were germinated into Vacin and Went medium *in vitro* from the three month old mature capsules. Subsequently the plantlet of this orchid species was grown into three solid culture media, i.e. MS, MSM and OMM, and they were successfully transferred from the lab to land condition. The Second orchid species, *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. was successfully grown in MS medium. The callus formation was observed from the young leaf and shoot tip of *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. But shoot tip has shown better response compared to young leaf. Therefore, after callus formation the shoot tip was considered to be the best explant for the production of plantlets of this orchid species.
From the above findings, it can be concluded that both vegetative and micropropagation are suitable for the production of offsprings or plantlets of all the plant species under study (i.e. *Homalomena aromatica* (Roxb.) Schott., *Smilax glabra* Roxb., *Bulbophyllum careyanum* (Hook.) Spreng. and *Paphiopedilum spicerianum* (Rchb.f.)Pfitz.). Among the propagation methods, micropropagation may be the only way for large scale production of the plantlets for commercial cultivation of the medicinal and aromatic plants to meet up the growing demand of the market. Moreover, the two propagation techniques may be successfully exploited for the conservation of different RET plants for sustainable use of these economically important bioresources. From the experience as described in the above it can be suggested that the topic of the present work has tremendous potential for further research on the propagation techniques of RET plants to enrich the biodiversity of this region at large.