STUDIES ON INTERACTION BETWEEN VAM FUNGI AND FRANKIA IN NON-LEGUMINOUS TREE SPECIES (ALNUS Sp.) OF NORTH-EAST INDIA

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CERTIFICATE

We certify that the thesis entitled "Studies on interaction between VAM fungi and Frankia in non-leguminous tree species (Alnus sp.) of North-East India" submitted by **Mahendra Pradhan** for the degree of **Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under our supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of Ph.D. degree. This work has not been submitted for any degree of any other university.

G. D. Sharma
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Place: Shillong

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GENERAL INTRODUCTION

Soil represents one of the most dynamic living entities which harbour a variety of microbes like Protozoa, algae, fungi, bacteria, actinomycetes etc. Some of the microbes in course of time have evolved from saprophytic to specialized symbiotic forms eg:mycorrhiza, *Rhizobium* and *Frankia* which are directly involved in plant nutrition. The genus '*Frankia*' (Lechevalier and Lechevalier, 1980) belongs to actinomycetes and is able to fix atmospheric nitrogen in root nodules of non-leguminous (actinorhizal) plants (Callaham *et al.*, 1978). So far 24 genera of woody non-leguminous angiosperms have been reported to form symbiotic association with *Frankia* (Dixon and Wheeler, 1986). Unlike legumes, actinorhizal plants however, possess a wide ecological tolerance which extends from temperate to subtropical and tropical zones of the globe. The potential biological nitrogen fixation by actinorhizal plants in forests has gained much attention in present days especially to minimize the use of costly inorganic nitrogenous fertilizers. Further, biological fixation of nitrogen by *Frankia* nodulated plants represent a substantial contribution to the global nitrogen cycle (Torrey, 1978) especially in the forests, wetlands and abandoned fields of the temperate and tropical regions of the globe (Bond, 1976) which is comparable to leguminous system in agricultural ecosystems. Besides, the actinorhizal plants have recently been increasingly exploited for reclamation of hostile wastelands, (Perinet *et al.*, 1985; Wheeler *et al.*, 1986), softwood production (Leikola, 1976; Gordon and Dawson, 1979; Pregnet and Camire, 1985), short term forestry (Gordon *et al.*, 1979), improving soil fertility (Tarrant and Trappe, 1971; Mikola *et al.*, 1983; Dawson, 1986), nurture plants (Thiagalingam, 1983; Turner *et al.*, 1978), wind breaks (Geary, 1983; Midgley *et al.*, 1983) and stabilization of lands (Silvester, 1977; Midgley *et al.*, 1983).

Under the natural conditons however, it is difficult to visualize a habitat in which both nitrogen fixing and vesicular - arbuscular mycorrhizal (VAM) microendophytes exist in isolation. In majority of the instances their ecological niche overlap depending upon the availability of nutrients to the host and consequently resulting into continuous interactions among themselves and host plants. Similar to legumes, the root systems of some actinorhizal species have been reported to form symbiotic association with VAM fungi (Williams and Aldon, 1976; Williams, 1979; Rose, 1980; Clelland ***et al.***, 1983; Gardner, 1986). ***Frankia*** and VAM fungi associated with non-leguminous plants ameliorate plant growth especially by supplying N and P to the host hence afford an ecological advantage over plants forming single symbiosis or without symbiosis. In fact, the plants forming tripartite associations are true pioneer species in colonizing nutrient-deficient and hostile habitats (Daft and Hacskaylo, 1976).

The role of mycorrhizal fungi in plant nutrition has received a great deal of attention which pertains to the mineral nutrition especially the uptake of non labile P. An adequate supply of P is important for satisfactory nodulation and nitrogen fixation by ***Rhizobium*** nodulated legumes (Demeterio ***et al.***, 1972) which may be accomplished by mycorrhizal symbiont (Carling ***et al.***, 1978). Besides, mycorrhizal fungi may also aid in the uptake of other elements like Cu, S, Zn and Mn (Starks, 1971; Mosse, 1973; Gray and Gerdemann, 1973; Mosse ***et al.***, 1976; Kothari ***et al.***, 1991) which may consequently lead to enhanced nodulation and nitrogen fixation by legumes (Crush, 1974; Daft and El-Giahmi, 1976; Smith and Daft, 1977). In non-leguminous system too, similar enhancement of nodulation, nodule activity and plant growth characteristics in response to VAM symbiosis under nutrient deficient conditions has been observed (Rose and Youngberg, 1981; Gardner *et al.*, 1984; Russo, 1989; Jha *et al.*, 1993). The tripartite association involving non-legume-*Frankia*-VAM fungi represents one of the most complex systems in which each microsymbiont interacts between themselves and with the host plant. No information is presently available on the seasonal nitrogen fixation by root nodules in actinorhizal system involving VA mycorrhizal symbiont in terms of *in vivo* morphology of microendophytes (*Frankia* & VAM fungi) which may add to present meagre knowledge on *in vivo* interaction between endophytes under field conditions where an array of limiting factors is operational.

Direct effects of *Rhizobium* and VAM fungi on each other are evident (Bayne *et al.*, 1984; Bethlenfalvay *et al.*, 1985; Brown and Bethlenfalvay, 1987) and exhibit both nutritional as well as non-nutritional, host mediated effects (Harris *et al.*, 1985; Brown and Bethlenfalvay, 1987; Brown *et al.*, 1988). Since the demonstration of importance of VAM fungi in nitrogen fixation (Asai, 1944) most of the interest in the VAM fungi-*Rhizobium* has gained attention in the role of former in the acquisition of P under P stress (Barea and Azcon-Aguilar, 1983). This role is clearly important and may influence nodule activity either through the enhanced nutritional status of the host plant (Cluett and Boucher, 1983; Carling *et al.*, 1978) or may directly or preferentially stimulate nodule function (Barea and Azcon - Auguilar, 1983; Van Nuffelson and Schenck, 1984). Further, the effect of *Rhizobium* on VAM fungi has been indicated (Pacovsky *et al.*, 1986) which may be mediated by production of polysaccharides by *Rhizobium* (Azcon-Aguilar *et al.*, 1980) or directly affecting metabolism of mycorrhizal fungus (Paccini *et al.*, 1988). However, in contrast to leguminous system, very little is known about the effect of VAM fungi on *Frankia* (Fraga-Beddiar and Tacon, 1990) and absolutely nothing on effect of latter on former.

Fixation of atmospheric nitrogen by actinorhizal tree species contributes a substantial amount of N to the forest ecosystems (Bond, 1967; Ferguson , 1971; Torrey, 1978) and a large proportion of latter is accreted to soil system by annual litter-fall (Tarrant and Miller, 1963; Zavitkovski and Newton, 1971; Debell and Radwan, 1978) thus contributing to existing soil organic matter (Tarrant and Miller, 1963; Bormann and Debell, 1981). This process may lead to elevated level of organic matter which may eventually influence the growth, survival, infectivity and effectivity of VAM fungi (Danielson and Visser, 1989) and *Frankia* microendophytes.

Besides, plant phenolics released from organic matter are known to affect *in vitro* growth and survival of both *Frankia* (Perradin *et al.*, 1983; Vogel and Dawson, 1985) and VAM endophytes (Pederson *et al.*, 1991). Presently no investigation is available on the effect of organic matter on growth, survival, infectivity, effectivity of endophytes and their interactions in tripartite association.

Soil pH has been known to affect survival and effectivity of symbiotic *Frankia* (Seilor and McCormick, 1982; Burggraaf and Shipton, 1982; Favre-Raynaud *et al.*, 1986; Smolander and Sundman, 1987; Smolander *et al.*, 1988) and VAM fungi (Kruckelmann, 1973; Graw, 1979). Further, the optimal pH requirement may also differ from microendophyte to microendophyte and between host plant and microendophytes. Effectiveness of tripartite association involving legumes may be severely affected by soil acidity (Newbould and Rangeley, 1984). But in non-legumes, studies in this field are lacking and warrant more endeavour. This is practically important as the supporting soil system is constamtly becoming acidic due to rapid replenishment of exchangeable cations with increasing soil N content (Crocker and Major, 1955; Debell and Radwan, 1983) and may exert harmful effect on the growth, survival and effectivity of mycorrhizal symbionts (Danielson and Visser, 1989) which is important in terms of nutrients uptake, nitrogen fixation and survival of host plants colonizing the degraded ecosystems. The increasing soil acidity may also influence growth, survival and nodulation by *Frankia* (Rodriguez -Barrueco *et al.*, 1968; Akkermans and Houwers, 1979; Smolander and Sundman, 1987) which is closely associated with N nutrition of pioneer actinorhizal species.

The importance of VAM fungi in P uptake by plants growing in infertile and high P-fixing soils (Mosse, 1973; Powell and Daniel, 1978) leading to improve plant growth is mainly due to capacity of mycorrhizal fungi to absorb and transfer P to host root beyond the depletion zone (Rhodes and Gerdemann, 1978; Li *et al.*, 1991). The ability of fungus in the uptake and efficient utilization of P from sparingly soluble (unavailable) rock phosphate sources under acidic conditions with consequential improved nodulation and nitrogen fixation by legumes is evident (Mosse *et al.*, 1976; Pairunan *et al.*, 1980). This is particularly important in tropics and subtropics in which many P-deficient soils occur in developing and underdeveloping countries where cost and rapid fixation of soluble fertilizers preclude their use. Besides, the increased application of soluble P may inhibit *mycorrhizal* colonization and spread thus affecting P nutrition which may be detrimental to nodulation, nitrogen fixation and growth of legumes (Asimi et al.,1980).

Therefore, the application of rock P may serve as excellent alternative to soluble P fertilizers in order to alleviate plant productivity. In contrast to leguminous system, studies involving non-legume-*Frankia*-VAM fungus symbioses, their interaction and overall effect on host plant growth under unavailable P source are unknown and warrant investigation which may be helpful in the future programmes on reclamation of nutrient-deficient habitats where P forms one of the major factor limiting plant growth.

North-Eastern region of the country which is rich in plant diversity is recently being threatened by continuous encroachment of forested areas, shifting cultivation (Jhum) and other human activities. However, the hills of the region are moist and favour development of variety of plant species among which actinorhizal species of ***Alnus nepalensis*** D.Don, ***Myrica esculanta*** Buch.-Ham.ex D.Don, ***Coriaria nepalensis*** Wall and ***Elaeagnus*** spp. grow luxuriantly on nutrient deficient soils and represent a dominant vegetation along with pine forest. ***A.nepalensis*** is more versatile, hardy and economically important species particularly colonizes hostile soil conditions of Eastern Himalaya. It fixes substantial amount of atmospheric nitrogen consequently improving N status of fragile forest ecosystem (Sharma and Ambasht,1984;

Sharma, 1988). However, investigation on characterization of two endophytes (*Frankia* and VAM fungi) of this region has not been accomplished although some information on VAM fungi from forested areas is available (Sharma *et al.*, 1986). These studies are important to alleviate productivity of actinorhizal tree species.

Keeping in view the above objectives, the present investigation has been carried out as outlined below :

- Seasonal study of microsymbionts (symbiotic *Frankia* and VAM fungi) morphology associated with seasonal changes in nitrogen fixation (nitrogenase activity) by *Alnus nepalensis* D. Don under field conditions.

- *In vitro* isolation, cultivation and characterization of *Frankia* and VAM fungal microendrophytes from root nodules and forest soils supporting *A. nepalensis.*

- Investigations leading to interactions between *Frankia* and *Glomus mosseae* under various levels of ;

1. Soil pH,
2. Soil organic matter and
3. Unavailable phosphate source (Rock phosphate)

and their effect on nodulation, nitrogenase activity, P uptake and growth of *A. nepalensis* seedlings under controlled conditions were carried out.