Chapter – IV

DISCUSSION
4.1 General

At the dawn of the new millennium, the evidence is to overwhelming that an agricultural transformation is essential to meet the challenges of feeding the growing population, conserving environment and reducing poverty. Such transformation is urgently needed in large agrarian economies such as India. An integrated natural resource management and precision farming applying all eco-technologies including integrated pest management (IPM), integrated nutrient supply system (INSS), crop improvement programmes, etc. are the requirement for such a transformation to meet the needs of our growing population without damaging environment and its ecological base.

Integrated pest management (IPM) is a component of sustainable agriculture and natural resource management with sound ecological foundations. Although the concept of IPM had its origin nearly three decades back, the use of pesticides and chemicals has not declined appreciably. Though the rate of pesticide consumption in India is low (300 g/ha) as compared to over 1 kg/ha in US and 10-12 kg/ha in Japan, several pesticide induced outbreaks have been reported from India in the recent past (e.g., Endosulphan issue). The indiscriminate use of broad spectrum chemicals has not only induced human health problems, but also resulted in the reduction in diversity of natural enemies, outbreak of secondary pests, development of resistance to pesticide by insects, pesticide induced resurgence and contamination of food and ecosystem. The increasing awareness and concerns about the horrors spawned with the use of chemical pesticides enhanced the demands for organically produced food crops and for avoiding the use of chemical inputs. Several safer pesticides and insecticides have been identified among which the botanical pesticides developed from plants have
found to be having greater potentiality in controlling pests and diseases. As many as 2121 plant species are reported to contain pest management properties, 1005 species having insecticidal properties, 384 having antifeedent properties, 297 having repellent properties, 27 having attractant properties and 31 have growth inhibiting properties (Singh, et al, 1996). The most commonly used pesticides are mainly derived from Neem (Azadirachta indica); Pongamia (Pongamia glabra) and Mahua (Madhuca indica). Currently several neem based commercial products are available in the market in different forms. Forty firms are now producing about 37 neem based pesticides valued at Rs. 300-400 million.

Neem, Azadirachta indica A Juss. (Meliaceae) is an evergreen, multipurpose tree, believed to be native to the Siwalik Deccan and other parts of South India and upper Burma. The tree is naturalized and grown widely throughout the country and is also growing in Sri Lanka, Indo-Chinese Peninsula and eastern Java to Sumbawe. During the last Century, because of its myriad benefits, the tree has been an ambassador of India and has migrated beyond the shores of the Indian sub-continent to many Asian, Caribbean and Mediterranean countries, Africa, Latin America, Australia and Philippines, among other countries (Fig. 1.1). Recently, it has been introduced to Saudi Arabia, Yemen, Philippines, USA, Brazil and Australia. Now it is well established in at least 30 countries world wide as a shade tree or as a component of agroforestry systems. An excellent discussion on the distribution of neem has been given by Dogra and Thapliyal (1993) and Dimetry (1993). In all these areas the tree is growing and thrives well in hot weather where the maximum shade temperature is as high as 49°C and tolerate cold up to 0°C. It is tolerant to soil alkalinity, salinity and acidity and grows well on all types of soils. This multipurpose
tree lives for 200 years and is growing well under tropical and subtropical conditions, semi-arid to wet conditions and from sea level to 1500 meter elevation. The natural or wild distribution of neem in a wider eco-geographic range indicates its adaptability from drier habitats to moist climatic regions Hegde (1996). Neem is presumably heterozygous and the cross pollinating behaviour along with the wider adaptability to varied ecogeographic range implies genetic variability offering a wide scope for selection of the required traits for improvement of the tree for more benefit. The choice of the most suitable genetic material for the improvement largely depends on the available genetic variability and high heritability of characters. Thus an understanding of the available genetic variability in the population and heritability of characters is the fundamental process and key attribute for the success of any improvement programmes. Over the past three to four decades the tree has come under close scientific screening world-wide as a source of natural pesticides, slow mineralising fertiliser, herbal medicine and several other products. Despite the widespread studies to find out various applications of neem parts, programmes for evaluation and improvement of the genetic resources of the species were not taken up much seriously, may be because of several constraints.

A large number of neem trees are found growing in Kerala and Tamil Nadu representing two major biogeographic systems, the wet-tropical and semi-arid conditions in India. The wide distribution of neem trees in the diverse environmental and ecological conditions in Kerala and Tamil Nadu implies its adaptability and genetic variability. The present study was undertaken to understand the genetic variability existing in the neem populations in Kerala and Tamil Nadu by evaluating the phenology, morphological characters of flowers, fruits, seeds, germination of
seeds and seedling characters in neem samples collected from eleven locations representing various eco-geographical zones.

4.2 Study locations

The present study aims at generating knowledge on the genetic variability and heritable characters existing in Neem trees growing in the diverse ecosystems of Kerala and Tamil Nadu in South India. The ecosystem, the basic fundamental unit of environment includes both the living and non-living components in an interacting way. The species in an ecosystem depend on each other and it is known that specific configuration of physical conditions (abiotic factors) influence the existence of these species. It is also well understood that the species in an ecosystem is governed by complex environmental factors and modification in both phenotype and genotype make-up may be taking place as a result of the stress from environmental factors like physiography, climate, soil, rainfall, etc. The study locations in Kerala and Tamil Nadu were selected by taking into consideration of these environmental factors and the population of neem trees. Thus, three locations were identified from Kerala and eight from Tamil Nadu, representing various ecosystem units, within the two broad biogeographic systems.

4.3 Exploration and population studies

The wide range of distribution of neem in various geographical locations is not recent and therefore, through natural processes appropriate selection must have taken place to adapt the species to survive under very diverse agroclimatic conditions. Considerable work has already been done on provenance trails for species having
wide range of distribution like *Eucalyptus commendulensis*, *E. terrestricornis*, *Acacia nilotica* etc., and substantial gains were obtained in their improvement. The population studies, exploration and collection of genetic materials and understanding the genetic variability existing in the population is the prerequisite in undertaking such studies. Hence, an extensive exploration and population assessment have been done in all the eleven selected ecosystems covering entire Kerala and Tamil Nadu state.

Although Neem is found all over in Kerala, it is not growing luxuriantly in all the places except Trivandrum and Palakkad region. It is found that, the density of population of trees were gradually decreasing from east to west, i.e., from Nagercoil region in Tamil Nadu to Trivandrum in Kerala and from Pollachi region in Tamil Nadu to Nenmara, Chittoor and Kodungalloor. Further, it is observed that the growth performance of neem trees in these places are good but in other locations of Kerala it is too poor when compared with Tamil Nadu. Most of the trees were appeared to be attacked by insect pests damaging the apical shoots and portion of the emerging branches. The canopy of the trees were found straight in most of the places mostly due to the lack of open canopy by the poor growth of other trees. The tree is a light demander though it can tolerate heavy shade during early years. This might have contributed for straightness of stem as a means to push it to get more sunlight. It is further observed that neem trees are more popular in areas adjacent to Tamil Nadu and Karnataka states particularly near the mountain passes such as Palghat gap, Bodinaikanur pass, Kambam pass, Aryankavu pass, Nagercoil pass, etc, and it may be assumed that the settlers from Tamil Nadu and Karnataka might have brought the trees to Kerala through these passes.
In Tamil Nadu, Neem is very popular and grows abundantly and luxuriantly in all the places, except the hill region in the west. It grows naturally as well as planted in households, public places, village gardens, protected compounds, roadsides and agricultural lands. However, population of trees in agricultural fields are comparatively less but are planted along the boundaries. Organised plantations were also found established in few places in Madurai and Tiruchirapally region. A gradual decrease in population from plains to the hill region was noticed and was completely absent above 1500 meters. In the hilly upland ecosystem zones the trees were noticed only at lower elevation. A sharp demarcation of the absence of neem trees in higher elevation has been observed in almost all areas of hilly uplands. The population assessment showed that the average population of neem trees per sq km is higher in dry southern ecosystem zone and lower in hilly upland ecosystem zone. More than 50 to 100 year old trees were very common in almost all the other ecosystems, particularly along roadsides and protected compounds.

4.4 Sampling tree selection

Selection of superior trees is the accepted and widely used approach and the fundamental processes in many tree improvement programmes. But it depends on the variability available in the population as a result of interaction of genes and their environment (Zobel and Talbert, 1984). The external appearance (Phenotype) and the variation in phenotype of a tree in any population is mainly due to the interaction of its genotype and environment. The distinct geographic or ecosystem with differences in edaphic and climatic conditions play a major role in the adaptive features of the plants in that particular ecosystem or environment. The selection of an outstanding or
superior tree as a sampling tree from its natural population of a particular ecosystem is the major approach in understanding the variability existing in the population.

Enough literature on heritable variations among individuals of a species and the importance of taking these variations as a basis for selecting individual trees for improvement programme are available. Heritability or heritable variation of a character in a population is the proportion of genetic variation to the total variation (genetic and environmental). This is affected by factors like age, location/ecosystem, climate and competition. The population of trees used for selecting superior trees in a given ecosystem is the base population. The base population may be of even aged or uneven aged. The growth efficiency of an individual tree of a given ecosystem is directly related to the degree of competition to which the tree has been subjected. The growth efficiency is indicated by its crown size and basal cover area of the tree. Further, the trees are annually subjected to a host of environmental influences such as drought, wind, and competition from adjacent trees, anyone of which could induce varying degrees of changes to the growth and reproductive behaviour of the trees.

The existing base population in the selected ecosystems in Kerala and Tamil Nadu were used for the selection of superior/sampling trees. Two methods were described in the literature for the selection of superior trees, one is Comparison method and other is Individual tree method. The Comparison or Check tree method is most vague and preferred for species growing in relatively uniform, even aged stands of a single dominant species or at the most only a few species. Under this three different methods are involved for the selection of superior trees. They are (i) Comparison tree method, (ii) Base value method, and (iii) Regression method. The first one is mostly followed for even aged stands of a single dominant species growing.
under comparatively uniform conditions whereas the last two methods are used for the stands, which are uneven aged and have high species diversity.

The individual tree selection method is used for stands, which are uneven aged. The candidate tree is evaluated for its values without making any comparison with base population values. Each trait of a candidate tree will be assigned some scores on the basis of its phenotypic performance. The values of these scores are used to select the candidate tree as a plus tree or to reject it. Three different methods were followed for selection of a plus tree under this general method and they are (i) Total score method, (ii) Independent culling method, and (iii) Selection indices method. Detailed description on the merits and demerits of all these methods have been discussed by Ledig (1974) and Sidhu (1996).

In the present study, the total score method under individual tree selection method has been followed for the selection of plus trees of neem growing under various ecosystem zones, since the trees in the base population are uneven aged and are under different management practices. This method of selection has been widely accepted in uneven aged populations of hardwood tree species and has been widely discussed by Zobel and Talbert (1984), Sidhu (1993, 1996, 1997). Minimum selection characters were fixed keeping in mind the economic importance and medicinal value of many parts of the tree and in relation to the main objectives of the study. It is given that the type of characters to be selected for the identification of plus trees is to be of inheritable value and are to be comparatively less affected by environmental factors. The more the number of characters, the response obtained for variability in individual traits may be less and therefore it is found desirable to select a few characters at a time like crown, stem, disease resistance, etc. Hence, the characters related to crown
and stem have been kept high followed by freedom from disease and insect pests, and thickness of barks for the selection of superior trees for sampling. The selected characters are DBH; crown shape and size; straightness of stem; bole with less stubs, burrs, bumping and straightness, diseases or pest incidence. Sampling trees have been selected in terms of the sum total of scores obtained by each candidate tree for the above individual traits. The candidate tree having a sum total of scores above the average of the area and having a superiority percentage of 20 and above have been taken as the sampling tree in that ecosystem for further studies. In the present study sampling trees have obtained more total scores than the candidate trees in base population of each ecosystem (Table – 3-14). The sampling trees have round shaped crown, straight bole and are devoid of any disease/pest incidence. The superiority in the characters (more than 25%) than the candidate trees have great significance and the present selection are expected to yield high genetic gain and heritability. The characteristics of sampling trees between various locations / ecosystems was varied mainly due to the age, and other environmental factors. But all the selected sampling trees are superior among the available population of neem trees in that ecosystem.

4.5 Phenological studies

Periodic behaviour or phenology of plants in tropical environment has received much attention in recent years. The phenological studies help in understanding the evolutionary dynamics as well as the reproductive biology of a species which in turn used in undertaking improvement programmes. Many species exhibit a strong rhythmicity in flowering, which is most prominent in tropical regions, mainly due to variations in climatic conditions. This may be seen in different populations of a single species whose natural range of distribution extends from
humid to dry climatic conditions. Rainfall, temperature etc, are found to be the trigger for flowering, flower initiation and abscission of leaves.

In tree improvement programmes knowledge of phenology is a pre-requisite and more recently efforts have been made to discuss the importance of general community pattern in leafing, flowering and fruiting of many species. A considerable amount of information is available on the major phenological events of plant species from different parts of tropical America, Africa, South East Asia, including continental India. Studies on phenology of trees in the forests of North Eastern India, Himalayas and in the deciduous forests of Western Ghats shed light on foliage dynamics, its relation to succession status of the given species, pheno-dynamics and the variation in the distribution of phenological events (Bhat, 1992).

In tropics, emergence of leaves is generally peaked either in dry season, or in the wet season, and leaf flush is attributed to the onset of rain after a spell of dry period, water stress, photoperiodism and temperature (Doubenmire, 1972; Proctor, et al., 1983; Whitmore, 1984; Prasad and Hedge, 1986). It has been accepted that environmental factors are to be favourable for maximising photosynthesis and vegetative growth, and the leaf abscission may be coincided with short day light and decrease in temperature (Sharma, 1970; Salisbury and Ross, 1974). However, the leaf-fall will occur for a wider span of time than for leaf emergence. The flowering in most of the trees were observed during the dry period and the advantage of dry season flowering may be that, it makes the flowers more visible to the pollinators, since the tree lacks leaves (Bhat, 1992). While there are extensive studies on the variation of phenological behaviour of different species particularly of forest ecosystem (Bhat, 1992; Murawski, 1995) detailed study on variation in the phenological behaviour
within a population of a single species growing under varied environmental conditions received less attention, may be due to difficulties in defining the traits because of environmental conditions, age and growth. However, such results is a prerequisite in understanding the variability existing in populations and in turn for improvement.

Isolated reports are available on the phenology of neem trees. Gogate and Gujar (1993) found that panicle with small honey scented white flower starts appearing in mid-March and ended by late April in a 50-60 year old plantation in Vidarbha region of Maharashtra. Other reports showed that flowering in Neem starts from January to March in Southern parts of India and late towards north (Anon., 1981). The flowering starts in the beginning of January in Kerala, in February-March in Karnataka, Tamil Nadu and Andhra Pradesh, in the first week of April in Central India and is getting progressively delayed in Northern India which may go up to the first week of May in the Sub-Himalayan areas (Anon., 1981; Tewari., 1992). Intermittent flowering and fruiting in Neem trees have also been reported from Murishidabad district of West Bengal (Guhabakshi, 1984). The pollen calendar of neem for six cities (Nair, et al., 1986; Datta and Chaturvedi, 1997) with respect to aeropalynology studies also showed that neem flowering is restricted from February to July in many parts of India.

The results on the flowering phenology of sampling trees of neem in eleven different ecosystems in Kerala and Tamil Nadu during the present study showed that the trees initiates flowering immediately after shedding of leaves along with flushing of new leaves. The flowering peaked during the months of January to May in all the location with variation among locations (Table -3.13). It is noted that generally the
flowering starts in December and ends by July. Peak flowering season also showed a wide variability in the 11 locations. In some locations a second flushing of flowers were observed during the month of September to November. The fruiting phenology of neem gave a similar pattern as in the case of flowering. The results of the foliage, flowering and fruiting phenology of neem trees are in confirmation with earlier reports and the variation between locations and between years may be due to difference in latitudes and other environmental conditions like rainfall, temperature and sunshine.

4.5.1 Flower and fruit bearing capacity

The factors determining the reproductive success in plants are a central goal for several studies in plant improvement programmes. It is recognised that fruit and seed production in an individual tree may be limited by either pollen or other resources allocated by plants to a flower in a particular season. An uneven distribution of resources may occur among different parts of plant or develop temporarily as the flower and fruiting season progress. In the present study, to understand the variation in flowering capacity and subsequent seed production, data was collected on the flower and fruit development in the sampling trees of all the eleven locations in Kerala and Tamil Nadu.

Flower bearing capacity studies revealed that the number and size of the inflorescence, number of flowers in a panicle and the distance between two adjacent panicles in a twig varied between locations (Table – 3.14). Maximum number of inflorescence (eleven) was observed at Chittoor and Thiruvaroor followed by Bannari and Tirunelveli. Size of the panicle interns of its length for the largest and smallest
panicle was found to have a wide range in its value and the minimum length for smallest panicles was recorded at Chennai and maximum for longest at Nagercoil. The distance between the inflorescences in a twig did not show much variability, which is evident from the narrow range of variation in the maximum and minimum distances between panicles of all the eleven locations.

The fruit development capacity of the sampling trees in various ecosystems in Kerala and Tamil Nadu showed considerable variation between location with higher percentage of fruit setting at Tirunelveli (34.1%) and lowest at Nagercoil (13.9) with a mean of 23.1 percentage. No correlation was found between number of flowers and number of fruits. The climatic conditions, physiological compatibilities of the flower, the availability and behaviour of pollinators and growth stages of the tree among others might have contributed for this variation between locations as reported by many workers (Murali, 1993).

4.6. Morphological studies

4.6.1 Floral biology

The study of reproduction in angiosperms or reproductive biology involves not only the transference of the male gametophyte, the pollen grains, to the female counterpart the stigma, through itself or with the help of mediators like wind, water, insect or birds but also the floral biology and floral presentation mechanism. The pollen grains, unlike the motile gametes can germinate and grow successfully on a very restricted place, the stigma of a compatible flower to produce pollen tube, which then grow through the style to the ovules leading to fertilization. In the pathway of the male gametophyte from the pollen to the stigma and from the stigma to the ovule there are manifold intricate devices to facilitate or to hinder self or foreign pollen or
gametophyte in attaining the goal. The flower, the fundamental reproductive unit of each species, get adapted to perform this function. The adaptations may be either positive or negative i.e. in the case of plants which crosspollinate with the help of animals or insects have an adaptation either to encourage the visits of particular animal and insect or to exclude the others.

In the tropics and subtropics flowers of most of the angiosperm plants are largely adapted to insect or bird pollination and the floral visitors consist of several species of insects and birds. Pollination of a flower by an insect takes place not in isolation but in a habitat of bewildering complexity and the flowers are evolved to serve this purpose. The establishment of flower-pollinator relationship is always dependent upon the presence of floral attractants like colour, scent, and availability of food source - the nectar and pollen. Owing to their early co-evolution with pollinator, flowers became an important factor in the diversification of certain animal groups and these animals in turn promoted the explosive radiation and diversification of flowering plants and thus the flowers were placed at the centre of this diversity.

The flower and all other floral attributes and the pollination behaviour are dynamic in nature and they are susceptible to climatic conditions. Slight variation in the environmental conditions throws the existing syndrome out of balance. An understanding of the floral biology is essential to appreciate the various events in reproductive biology as well as in any plant improvement programme. It is important to quantify a variety of morphological traits of the flower to obtain an insight into the nature of selective pressure that have generated in the floral morphology and in turn understanding the variability existing in the population through the selective pressure from environmental factors (Ornduff, 1969; Murawski, 1995).
Ever since the pioneering classical work by C. K. Sprengel (1793) the study of floral biology has diversified into many branches of botany and in interface with zoology. Floral biology studies now embrace morphology, anatomy, physiology, development, pollination biology, breeding systems, genetics, molecular biology, paleobotany, diversity and evolution. During the past few decades with the elaboration of scanning electron microscopy, and with the advent of new molecular genetic technique and broader diversity studies contributed much to floral biology by giving fascinating information.

Neem with its unattractive white or pale yellow flowers with characteristic sweet smell is the most familiar economically important tree of the tropics with multivariant uses. Flowering in neem normally starts at the beginning of the dry season and continue to flower for 3 to 5 months. The flowers are cross-pollinated and fertilisation is porogamous along with syngamy and triple fusion. Although it regularly produce flowers, fruits and seeds, not much is known about its floral biology including the variability existing in flower morphology at various ecologically distinct locations. There are only a few scattered bit of information available about the flowers, but these available information did not give a coherent understanding of the variability available in the population located in diverse ecosystems. The floral biology and embryology of Neem flower has been studied by Garudamma (1956, 57); Murthy and Gupta (1978); Nair (1956); Nair and Kanta (1961); Nair et al., (1986) and Dayanand, et al., (1993). They described flower of neem as bisexual, pentamerous and bracteate. The bracts are minute and deciduous. The flower consists of 3 to 5 free sepals with imbricate or valvate aestivation and corolla with 5 oblong, widely spreading and spatulate petals showing imbricate or quincuncial aestivation. Stamens
numbering ten with filaments united to form a monoliform tube, which is situated at the base of the hypogynous disc. The ovary of neem flower is tricarpellar, syncarpus and superior, trilocular at the base and unilocular above. The ovules are present in each locule on parietal placentation. The length of the style is equal to that of the staminal tube. The style tip is expanded to form a ring bearing three acute partially fused papillose stigmatic lobes. Pollen grains are trapped and germinate in this region. The mature pollen grains are tri or tetracolporate, prolate, spheroidal or sub-prolate. Apocolpium is medium and endocolpium is la-longate and elliptical. Ora is circular. The exine is psilate and the size of the grain is 37 x32 µm. The above basic information on the floral biology has been used to standardise the various parameters and methods for the study of variability in the flower morphology of neem existing in different ecosystems in Kerala and Tamil Nadu.

Flowers were collected from various branches of the sampling trees, selected in all the 11 locations observed and measurements were taken. The data on sepals, petals, androecium and gynoecium were noted for all the sampling locations, average values were computed and is given in Table 3.15 to 3.17. In general the present observation are in agreement with those of Nair (1962, 1963); Murthy and Gupta (1978) and Dayanandan, et al., (1993). However, number, shape, arrangement and characteristics of floral appendages showed variation between sampling locations (Table – 3.15). It is observed that the bracts were present in all the locations, however it is found absent in the samples collected from Sengottai. In the case of calyx variations between locations were observed for number, shape, size and arrangement. In all the locations the number of calyx was five but in the samples from Nagercoil and Sengottai only 4 and 3 sepals were noted. The calyx in the Trivandrum samples
had obovate in shape whereas in other locations it is the oblong. The arrangement of calyx was imbricate in seven locations, however valvate arrangement was noticed in one location, namely Nagercoil. Both imbricate and valvate aestivation is noticed in three locations namely Namakkal, Thiruvaroor and Sengottai. Except in size and arrangement, no variation is found in corolla between locations. In seven locations imbricate aestivation was noticed and in three locations quincuncial aestivation was noted. Both imbricate and quincuncial aestivation was noticed in the samples collected from Thiruvaroor. Imbricate and valvate aestivation was reported by Nair (1962) in the arrangement of sepals and petals, however Murthy and Gupta (1978) reported quincuncial aestivation also in the petals of *Azadirachta indica*. Among the characters of androecium, number of stamens and lobes, shape and aperture of pollen grains and density of hairs in staminal tube showed variation between locations other than size. Except Nagercoil, flowers from all other locations showed 10 number of stamens and lobes. This observation is in confirmity with the report of Murthy and Gupta (1978) where they also noticed 10 lobes and anther in *Azadirachta indica*. In the present study both prolate and spheroidal shaped pollen grains with tri and tetra zonocolporate asperture is noticed. Spheroidal shaped pollen grains were observed in three locations (Thiruvaroor, Nagercoil and Sengottai) and Trizonocolporate pollen grains were noticed only in Thrithelloor samples, but both tri and tetra zonocolporate pollen grains were noted in the samples collected from Thiruvaroor, Tirunelveli and Sengottai. The presence of hairs in staminal tube also showed variation particularly for its density. Density of hairs was high in the samples collected from Sengottai, whereas in the samples collected from Thrithelloor, Thiruvaroor and Nagercoil medium density of hairs were noticed.
In the case of gynoeicum not much difference is noted except in the character of stigma, placentation and the size of appendages. Both papillose and baculate type of stigma were noticed in eight and three locations, respectively. Parietal placentation was common in all the locations, however both axial and parietal placentation were noticed. Nair (1956), Murthy and Gupta (1978) also reported parietal placentation in neem.

4.6.2 Fruit and seed studies

Fruits and seeds are the distinctive features of angiosperm. Biologically fruits are developing from ovary and seeds from ovule. In neem, among the six ovules one develops into a seed. The seed is composed of a shell and a kernel. The seed is normally ovoid or spherical pointed above and has a thin testa (Pennington & Styles, 1975). The fruit of neem is a smooth, ellipsoidal drupe, and green when young and become yellow or brown when ripe. The epicarp is thin and endocarp is hard and bony. The mesocarp is pulpy and is eaten by birds and animals. The green mature fruits are almost 2.0 cm long (Mohan Ram & Nair, 1993). The neem is usually propagated by seeds and seedlings raised in nursery and the availability of better quality seeds are prerequisite in plant improvement programmes. The successful regeneration of plants depends on quality of seeds, their viability and vigour which ultimately depended on the physiology and environmental conditions. It is reported that the seed size and weight often controls the germination and initial seedling growth in plant species (Langdon, 1958; Wood, et al., 1977; Kandya, 1978; Quraishi and Mishra, 1996; and Nizam and Hossain, 1999). In the present study important fruit and seed characters like number, shape, size and fresh and dry weight of fruits and seeds collected from all the eleven locations were studied to understand the nature and
extent of available variability in fruit and seed characters (Table 3.18). Considerable variations were found for the development of fruits between locations. Average number of fruits developed varied from a minimum number of 5 in a single panicle to a maximum number of thirteen. Both ovoid and oblong shaped fruits were observed in five and 4 locations, respectively. In two locations both oblong and ovoid shaped fruits were noticed. Considerable differences in size and weight of fruits were also noticed between locations.

In the case of seeds the average length was found to vary from a minimum of 2.0 cm for the seeds collected from locations 3 and 5 to 2.8 cm from the location ten. Seed length in 5 locations were higher than the general mean length (2.3 cm) recorded. Breadth of the seeds in all the locations also had a range of values varying from a minimum of 1.2 cm to a maximum of 1.7 cm. The average among all the locations for this parameter was 1.5 cm. The size of the seeds as defined by its L/B ratio was higher than the general population mean in 5 locations, namely Thrithelloor, Bannari, Nagercoil, Sengottai and Tirunelveli.

The seeds collected from the eleven locations were also characterised for their average fresh and dry weights. Maximum fresh weight was recorded for the seeds collected from Nagercoil and the minimum value was recorded for the seeds collected from Tirunelveli. This is in agreement with the varied seed length and breadth values recorded for the genotypes from these locations. The dry weight of seeds recorded an average value of 25.4 g and it is noted that even though the fresh weight is lowest in the seeds collected from Tirunelveli, the dry weight of these seeds were comparatively high. This shows the influence of climate particularly rainfall and temperature on seed weight as Tirunelveli and Nagercoil locations experiences
entirely different climate during the fruiting season. A close relationship between seed size/weight and seed quality has been documented for many tropical hardwood trees and (Acquiare and Nakare, 1983; Gupta, et al., 1983; Halos, 1983; Srimathi, et al., 1991; Ponnammal, et al., 1993) reported that medium-sized seeds performed better than the large size and seeds. Jenner, et al., (2003) observed distinct variation in seed characters like seed length, seed breadth, length-breadth ratio, seed weight etc. among the 23 one present families of Madhuca latifolia (Roxb) collected from different agroclimatic zones in Tamil Nadu.

4.7. Germination and seedling studies

4.7.1 Seed viability and germination

Seed viability is of considerable importance in any crop improvement programmes. The hybrids can be successfully produced in any conventional breeding programmes only if the hybrid seed can be successfully germinated into a vigorous plant. The successful regeneration of plants depends on quality of seeds their viability and vigour. The use of proper seed source is the backbone of any tree improvement programme and seeds must be genetically superior to produce good seedlings. In the case of neem the viability of seeds is very short and seeds can be normally stored for three months provided they are properly processed (Nagaveni, et al., 1987).

Considering the importance of viability of seeds, samples of seed were collected from all the eleven locations and tested and compared (Table - 3.19). The data revealed significant difference between the locations. It varied from a minimum value of 304 seeds (76.0%) in Bannari to the maximum of 96.0% for the seeds collected from Namakkal and Chittoor. This brings out the existence of the inherent genetic
variability in the population, which is of great benefit in selection programmes by applying necessary selection pressure for this character during improvement.

Most of the variability studies in neem were carried out to understand the storage life and found that storing of seeds with high moisture content in deep freeze condition is having deleterious effect on seed viability. (Gurudev Singh, et al., 1997). The nature of the seed, like chemical composition, seed coat characteristics and genetic composition of each species is responsible for its longevity under identical storage conditions. Bauhenia purpurea, Butea monosperma, Cassia glauca, Caesalpinia coriraria, Leucaena glauca, Mimosa pudica, Peltoforum ferruginium, Pithecollobium dulce and Prosopis julifera remained viable after the storage of 6-7 years. The longevity of seed viability varies from taxa to taxa and even species to species under the same packing and storage conditions as obtained in Albizia lebek (Sahai, 1999)

The germination percentage of seeds, another character of great importance in the context of improvement programmes was observed both under normal condition as well as after treating with chemicals like Thiourea and Gibberellic acid. The maximum germination of 93.3% and minimum 30.0% was noted for seeds collected from Thrithelloor, Chittoor and Chennai, respectively. The average among all the locations studied was 58.5%. Mohan Ram and Nair (1993) also reported higher percentage of germination for neem seeds and the germinability can be improved by soaking. Aiyadurai (1959) reported that germination differs depending on shape i.e. elongated or spherical. The application of Thiourea and Gibberellic acid did not show any significant effect on germination or the speed of germination during the present
study. However, Chaney and Knudson (1988) reported that the removal of endocarp enhanced the germination capacity of neem seeds.

Variation in germination percentage in seeds obtained from different states in India has been reported in *Acacia* by Dobryal and Bagchi (1990) and in *Pinus* by Zobel (1990). Location wise variation in seed parameters and germination was reported (Tyagi et al, 1999). Shivakumar and Banarjee (1986); Chauhan and Raina (1980); Nizam and Hossain, (1999) noted that bigger sized seeds and increased seed weight shown better and uniform viability and germination. The seed size often controls the germination and initial seedling growth in plant species (Kandya, 1978; Langdon, 1958; Wood, et al., 1977) and found that usually bigger seeds germinate faster and attain enhanced growth than the smaller one (Whuang Plaong, et al., 1994; Quraishi, and Mishra, 1996).

4.7.2. Seedling studies

4.7.2.1 Morphology

Seedling is the juvenile stage of the plant produced from seeds. On germination majority of dicotyledons conform to two of several patterns of development like the cotyledons are either extruded from the testa (Phanerocotylar) or they remain closed cryptocotylar, otherwise the cotyledons are raised above the soil surface (epigeal) or remain in the soil (hypogeal). The dicot seedlings mainly consists of root, hypocotyl and a plumular bud with subsequent internodes and eophylls. All these parts may be well developed or present in more or less reduced form or even entirely absent. The seedlings sometimes resemble or differ in varying degrees from adult plants of the same species. Several workers have made use of data on seedlings in taxonomic work, but it has also got profound importance in the maintenance and
conservation of plant diversity either directly or indirectly as well as in the improvement programmes of economically important plant species. The data on seedling derives primarily on observable characters like number, form, size and shape of cotyledons and the form, size, shape and phyllotaxy of the earlier leaves, eophylls. The ultrastructural features of cotyledons and eophylls are also considered (Paria, 1997).

In the present investigation, seedling raised from seeds collected from all the eleven locations in Kerala and Tamil Nadu were compared for the behaviour in germination, number, colour, shape and size of cotyledons and the size, shape and phyllotaxy of eophylls. The growth behaviour of seedlings raised from both treated and untreated seeds were also noted (Table 3.21-3.25).

It is observed that the germination is epigeal and phanerocotyler for the seeds from all the locations and no variation is noticed in the number, shape and colour of cotyledons and also the phyllotaxy and shape of eophylls. The radicle emerges from the seed, withdrawing cotyledons from the coverings and in straight raising them above ground. The primary root is moderately long and tapering, lateral roots are moderate in number and length, fibrous and distributed down the main root. The hypocotyle is distinct from root, terete, cylindrical, white initially and turning green glaborous soon. Cotyledons are two in number, thick, fleshy, elliptical, apex rounded, outer surface convex, inner flattened (outer planoconvex) entire and in creamy white colour when young but green and glaborous later. The eophylls are opposite, trifoliate and deeply lobed. In the first stages the seedling are not resembling to the present but after 3 or 4 leaves onward leaves become compound with leaflets.
Considerable variation in size of cotyledons and eophylls were noticed between locations may be due to the variation in seed sources, size and weight of seeds.

4.7.2.2 Seedling growth

Studies were carried out with a view to assess the variability in the growth of seedlings raised from seeds collected from the sampling trees representing eleven agroclimatic zones in Kerala and Tamil Nadu. The data revealed distinctive variation among the seed sampling sources in respect of root length, number of lateral roots, shoot length, collar diameter and fresh and dry weight of both root and shoots. The effect of chemical stimulants like thiourea and gibberellic acid at different concentrations on the above parameters also showed considerable variation between sampling locations. In a few locations thiourea showed little effect at a certain concentration and gibberellic acid also showed its effect in certain locations at certain concentrations. Thus the seedling growth as well as the effect of chemical stimulants on seedling growth varied between locations to locations in neem. This shows that the genotype of the present plant (seed source) has a strong effect on the seedling growth characters. Similar results were obtained in neem for Gera, et al., (1998) in the study on rooting response of shoot cutting of different provenances. Kumaran, et al., (1991) reported variation in number of leaves, basal diameter and total dry weight. Information on such variable is available on other tree species like Pongamia pinnata, Acacia nilotica, Eucalyptus camendulensis, Terminalia pinnata (Padmini and Banerjee, 1986) and Santalum album, (Manojkumar, 1994). The selective pressure, genetic drift and other factors might have contributed for such variation as noted by Jenner, et al. (2003).
4.8 Statistical analysis

4.8.1 Genetic variability

The understanding of available natural genetic variation within a population existing in different agroecological condition is the first step in any improvement programme. A tree breeder selects superior genetic material (seeds or clones) from the species natural range or from other sources. The distribution range of neem is known precisely, however studies on genetic variation in its ecogeographic range of occurrence have not been carried out in its areas of natural origins. Neem (Azadirachta indica) is a perennial tree, presumably heterozygous and is cross pollinating. This implies genetic variability offering a wide scope for selection of the required traits for improvement. Though sufficient information is available to consider neem as a "wonder tree, or a tree for solving global problems", very little work has been reported in the collection and evaluation of germplasm material for the benefit of improvement. In the present investigation the pattern of intraspecific variation has been studied and evaluated for superior germplasm selection for improvement work. The morphological characters of flower, fruit, seed and seedlings has been placed at highest level in the evaluation of the variation. The selection of superior germplasm is the most popular method in the improvement of several tree species like rubber, eucalyptus, acacia, etc. The variation among the eleven populations identified in the various agroecosystems in Kerala and Tamil Nadu was assessed through the analysis of variance, estimating the phenotypic and genotypic coefficient of variation and the heritability of the selected morphological characters through statistical methods and is discussed below under various headings.
4.8.2 Analysis of variance

Analysis of variance was carried out for the characters of flower, fruit seed, seedling revealed very high significant genetic variation at 1% level of probability for most of the characters among the eleven locations studied (Table – 3.16). The results revealed the presence of inherent genetic variability in the population which will enhance the selection programme and enable the worker/breeder to apply enough selection pressure for these characters. Considerable amount of variability for many of the floral characters showed richness of genetic diversity existing in this economically important species. Among the floral characters anther length and pollen size did not show much variability. The data on fruit length and diameter when subjected to analyse of variance found that the genotypes in these eleven location had statistically significant differences. ANOVA was also carried out for the seed characters like length, breadth, fresh weight and dry weight. Statistically significant difference were found for all the seed characters studied including the inherent genetic variability in these characters. Provenance variation in seed parameters and germination was reported by Tyagi, etal, (1999) and Shivakumar and Banarjee (1986) Chauhan and Raina (1980). And showed that bigger seeds reflected better and uniform viability and germination. In general, the analysis of variance carried out for 27 characters of flower, fruit, seed, seed germination, seedling and growth characters of seedling both control and after treatment revealed very high significant genetic variation at 1.0% level of probability for all characters except two among the eleven location studied. The results revealed the presence of inherent genetic variability in the population, which will enhance selection programmes and enable the breeder to apply enough selection pressure for these characters. Kumaran, et al., (1993) reported 1 per cent
level variation in seed parameters among the 28 one-parent families of *Azadirachta indica* collected from Tamil Nadu. Similar results in variability studies were reported by Bagchi and Sharma (1989) for *Santalam album* and Squillace (1966) in Slash Pine.

4.8.3 Genetic parameters

In most of the tree species, the characters display quantitative variation through the joint action of a number of supplementary genes each having a small effect in relation to total variation, which is termed as a polygenic system (Mathur, 1941). In tree improvement programmes, the knowledge of the polygenic characters and the genetic variation available in any population is a pre-requisite (Mathur and Jinks, 1977) but it is not measurable directly. Only phenotypic characters, the external expansions of genetic values modified by environment is measurable. The application of comparing data on phenotypic characters between different population for understanding genetic variability have been demonstrated in coconut by Shrikhande (1957). Later Gilbert, *et al.*, (1973) reported on the application of these analysis for the study of Rubber progeny data.

Genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability in the broad sense ($H^2$) help in partitioning the genetic variability into heritable and non-heritable components (Johansen, 1909; Henderson, 1953; King and Henderson, 1954; Singh and Chaudhary, 1979). While the phenotypic coefficient of variation measures the extent of total variability for a character, the range of genetic diversity for quantitative characters in a population can be compared and assessed reliably by genotypic coefficient of variation. Burton (1952) had suggested that genotypic coefficient of
variation together with heritability estimates would give a better idea regarding the amount of genetic advance to be expected by selection. Selection acts on genetic differences and gains from selection for a particular character depends largely on the heritability of that character (Allard, 1960). Several people have discussed several methods for estimation of genetic parameters in perennial economically important tree species including forest species (Kedarnath, et al., 1960; Sakai and Hatakeyama, 1963; Sakai and Mukhaide, 1967; Sakai, et al., 1971; 1963 & 1967; Huhn, 1975; Abraham, et al., 1992; Mydin, et al., 1996).

In the present study coefficients of variation at the phenotypic and genotypic levels and heritability in the broad sense ($H^2$) were worked out for 14 floral characters, 12 fruit, seed and seedling characters and 8 growth characters of seedlings under control and after treatment (Table 3.29 – 3.32). For all the characters studied except a few like corolla length, anther and pollen size, the phenotypic coefficient of variation was always higher than the genotypic coefficient of variation. This is only natural as variability at the phenotypic level consists of both genotypic and environmental variabilities, besides the genotype-environment interaction ($G \times E$). When the total variability for any character is contributed mainly by the genotypic variation and is less influenced by the environmental factors the scope for selection of this character increases.

Phenotypic coefficient of variation was found to be the highest for the width of the staminal tube at the middle region (28.39) followed by width of staminal tube at the tip (22.39). These characters with their medium to high phenotypic coefficient of variability demonstrated the high amount of variability as a result of the total of
genotypic and environmental contribution. Very low PCVs (Table – 3.29) were recorded for length of staminal tube (6.64) and style (7.02).

Genotypic coefficient of variation was the highest for width at the middle of the staminal tube (28.05) followed by width of the staminal tube in the tip region (21.89). The medium to high estimates of coefficient of variation at the genotypic level is a measure of the variability expressed due to its genetic characteristics. Very low genotypic coefficients estimated for the characters like length of the staminal tube (5.99) and style (6.42) depicts the lesser extent of variability in these characters even at the genotypic level. For all those characters with medium to high estimates of phenotypic and genotypic coefficients of variation demonstrates the wide variability expressed for those characters. The differences between the PCV and GCV for most of the characters were very low indicating the significant involvement of genetic factors and the negligible influence of the environment in the expression of these characters. The maximum difference between the two coefficients was noted for the length of the corolla, which indicates a comparatively higher influence of the environment in the expression of this character. The narrow differences between the phenotypic and genotypic coefficients of variation for the studied characters are indicative of the comparatively stable nature of these characters due to the negligible influence of the environment on them. The medium to high estimates of variability recorded suggests the possibility of genetic advancement for these characters through selection.

Heritability of a character specifies the proportion of the total variability that is due to genetic causes, or the ratio of the genetic variance to the total variance (Allard, 1960). Lush (1937) has defined broad sense heritability ($H^2$) as the proportion of total
genetic variance to the total or phenotypic variance. It refers to the functioning of the whole genotype as a unit and is used in contrast with environmental sources of variation (Lerner and Michael, 1950). The estimation of heritable variation is not possible with the help of genotypic coefficient of variation alone. Burton (1952) suggested that genotypic coefficient of variation together with heritability estimates would give a better idea of selection advance to be expected. Selection acts on genetic differences and gains from the selection for a specific character which depends largely on the heritability of that character (Allard, 1960). Broad sense heritability reflects the proportion of additive plus non-additive genetic variance and is useful in predicting improvement achieved by cloning selected trees (Hogarth, 1971).

In the present study, it was found that all the floral characters except two had high estimates of broad sense heritability. Broad sense of heritability at higher level was estimated for the width of the staminal tube at middle region (98.99%) where the difference between PCV and GCV was the least, followed by width of staminal tube at the tip region (95.59%). Medium estimates of broad sense heritability were noted for the character, length of the anther (45.37%). It can be seen that all those characters with medium to high estimates of broad sense heritability are largely under the genetic influence rather than environmental influence and has a very high potential of inheritance.

Fruit, seed and seedling characteristics in all the locations were analysed and the results are shown in Table 3.30. Phenotypic and genotypic coefficients of variation and broad sense heritability estimates showed clear evidence about the
existence of a large amount of variability influenced strongly by their genetic makeup rather than the environment as indicated by the narrow difference between PCV and GCV estimates. However, in few characters like fruit and seed breadth, seed viability and cotyledon length showed broad difference between PCV and GCV which reflects slightly increased influence of environment in these characters. The high and medium heritability estimates in the broad sense for some of the characters showed that these characters are also highly heritable to the next generation. Kumaran, et al., (1993) also recorded moderate GCV and PCV for seed length and breadth and for the weight of 100 seeds along with moderate to high heritability for seed weight and seed length, which is almost identical to the present result. Bagchi and Sharma (1989) also shown higher GCV for seed weight and seed length in Santalum album.

PCV and GCV and the heritability in broad sense were also analysed for the growth character of seedlings. They are length of root and shoots, number of lateral roots, collar diameter, fresh and dry weight of roots and seedlings both in control and after treatment. It is found that the PCV was always higher and the difference between PCV and GCV for almost all the characters studied did not show much difference, which indicate a uniform influence of genetic and environmental factors with external factors having slight edge. Heritability in the broad sense was found to be high for all the treatments for almost all the characters, indicating high heritable nature of these characters within the existing variability.

4.8.4 Genetic divergence

Genetic divergence in a population, especially in respect of the characters in which improvement is sought, is an indispensable pre-requisite for
successful crop improvement programme. The D^2 statistics has found as a favourable tool for estimating genetic divergence, which is the basis in choosing parents for hybridization. This is well illustrated in several crop plants (Vairavan, et al., 1973; Bavappa and Jacob Mathew, 1982). Progenies derived from diverse crosses are expected to show a broad spectrum of genetic variability providing greater scope for isolating high yielding segregates in the succeeding generations. Thus genetic divergence is an important and indispensable prerequisite in any successful crop improvement programme, especially in respect of the characters in which improvement is sought. Incorporation of superior genes identified and isolated from the highly potential germplasm could then be achieved by hybridization between highly divergent genotypes, which can result in very high heterotic effects.

Selection of parents on the basis of their genetic distance helps in a better realization of heterosis, but desirable and high magnitudes of heterosis are not directly related to extreme parental divergence as discussed by Arunachalam, et al., (1984) and Mydin (1992). Employment of D^2 analysis successfully has been reported in a number of perennials and annuals like Banana (Valsalakumari, et al., 1985); Sugarcane (Punia et al., 1983 and Ram and Hemaprabha, 1993); Coconut (Balakrishnan and Namboodiri, 1987) and rubber (Markose, 1984; Mydin, 1992; Abraham, et al., 1995, 2000). In the present study, genetic divergence was estimated between the neem trees selected over eleven agroclimatically different geographically distinct locations using the Mahalanobis D^2 statistics. The eleven locations were statistically clustered into six genetically divergent clusters (Table – 3.34) using Mahalanobis D^2 statistic based on the D^2 values depicted in Table – 3.33. Majority of the locations were grouped in clusters one and six. Cluster number one had the
maximum number of locations (Four) and cluster numbers two and six, immediately following, accommodating two locations each. Three clusters, namely 3, 4 and 5 had only single locations grouped under them.

The inter and intra cluster distances were also estimated based on $D^2$ values. The maximum intercluster distance (1045.28) was observed between clusters 4 and Cluster 5 followed by clusters 2 and 3 with an intercluster distance of 507.51 (Table 3.35). Parents with intermediate genetic divergence also have a higher chance of producing heterotic hybrids (Thakur and Zarger, 1989). Selection of parents will hence be more appropriate from those clusters separated by intermediate to high genetic distances (Mydin, 1992) because strong positive relationship have been found between genetic distance and heterosis in a broad range of crop species (Balesch, et al., 1984; Shamsuddin, 1985). Thus it can be seen that all locations had medium to high intercluster distances indicating the genotypes in each locations to be a suitable parents with genotypes in other clusters.

Clusters 3, 4 and 5 had only single location with their intracluster distances being zero. Other clusters viz., 1, 2 and 6 had their intracluster distances 54.61, 121.06, and 114.06, respectively. Thus it is evident that there is a great scope for identifying suitable parents in the present sample of wild germplasm of neem studied.