PROVENANCE TRIAL OF *PARKIA TIMORIANA* (DC.) MERR. IN NORTH-EAST INDIA

UTTAM THANGJAM

DEPARTMENT OF FORESTRY

MIZORAM UNIVERSITY

PROVENANCE TRIAL OF *PARKIA TIMORIANA* (DC.) MERR. IN NORTH-EAST INDIA

By

Uttam Thangjam

Forestry Department

Submitted
in partial fulfillment of the requirement of the degree of Doctor of Philosophy in forestry of Mizoram University, Aizawl.

DECLARATION

I, Mr. Uttam Thangjam, do hereby declare that the thesis entitle "Provenance trial of *Parkia timoriana* (DC.) Merr. in North-east India" is the record of original work done by me under the supervision of Dr. U.K. Sahoo, Professor, Department of Forestry, Mizoram University. The thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and it has not been submitted by me for any research degree in any other University or Institute.

This is submitted to the Mizoram University for the degree of Doctor of Philosophy in Forestry.

Date:

Place: Aizawl (Uttam Thangjam)

(Prof. U.K. Sahoo)
Supervisor
Department of Forestry
Mizoram University

(Prof. B. Gopichand)
Head
Department of Forestry
Mizoram University

Mizoram University

Department of Forestry

School of Earth Sciences and Natural Resources Management,

Aizawl – 796004

CERTIFICATE

This is to certify that the thesis entitled "Provenance trial of *Parkia timoriana* (DC.) Merr. in North-east India" submitted by Shri. Uttam Thangjam, for the award of the degree of Doctor of Philosophy in Forestry of Mizoram University, Aizawl, embodies the record of original investigation carried out by him under my supervision. He has fulfilled all criteria prescribed by the UGC (Minimum standard and procedure governing Ph.D. regulation), including the mandatory publication (publications enclosed). It is also certified that the scholar has been admitted in the department through an entrance test followed by an interview as per clause 9 (i) & (ii) of the UGC regulation 2009. His thesis presented is worthy of being considered for the award of the Ph. D. Degree. The work has not been submitted for any degree to any other University.

Date:

Place: Aizawl

(Prof. U.K. Sahoo)
Supervisor
Department of Forestry
Mizoram University

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CHAPTER 1

General introduction

Parkia timoriana (DC.) Merr. commonly-known as tree beans or stink beans is an agroforestry tree species of tropical region. The tree grows luxuriantly in wild, in home gardens and in shifting agriculture lands of North-east India (NEI), particularly Manipur, Nagaland, Meghalaya, North Cachar Hills and Sibsagar districts of Assam (Kanjilal et al., 1982). It is one among the 31 species of the genus *Parkia* reported to grow in Africa, America and Asia. Hopkins (1994) describes 10 accepted taxon of this genus which occurs from NEI to Fiji and they appear to be more similar to African species than the American ones. Hooker (1897) reported four species of *Parkia* from India, however recently Angami (2018) reported 6 species of this genus, namely *P. timoriana* (DC.) Merr., *P. filicoidea* Oliv., *P. biglandulosa* Wight et Arn., *P. clappertoniana* Keay, *P. bicolour* A. Chev. and *P. speciosa* Hassk. There are altogether 39 accepted names and 6 scientific names of infraspecific rank for all the plants under the genus *Parkia* (www.theplantlist.org).

Tribal communities of NEI were found to domesticate various wild plants for its palatability and medicinal properties. *P. timoriana* is important to the community mainly for an economic and nutritional reason. Starting from flowering till pod maturity, every stage of *P. timoriana* could be consumed to give a different flavour. Their traditional cuisines made from the pod and seed of this species have an important place among other dishes to be served on various rituals and occasions. Other uses of this species are anti-diabetic, antioxidant, pulpwood and firewood (Angami, 2018). People of this region also use sundried exocarp as a medicine to cure stomach problems. In addition, the tree is associated with various belief and culture among different communities of this region. People of the ethnic communities in Manipur were found to wrap the stem of the tree with a piece of indigenous women clothing. This is done to trees with age more than 7 to 8 years and but does not bear flower and fruit. By doing so it is believed that the tree would flower and eventually bear fruit. In contrary, the utilization and consumption of this tree species have not been fully explored in other parts of India. This may be because of the pungent smell of the fruit produced by a sulphur-containing amino acid (Thiazolidine-4-carboxylic acid). This tree species is also widely distributed in Asia-Pacific regions. It is very popular in southern Thailand, Myanmar, Malaysia and Indonesia where they used the seeds for cooking with duck or eat with other flavouring agents such as shrimps. Besides human, the seeds are also found consume by other mammals such as langurs, hoolock gibbon, slow loris and birds such as large green billed malkoha, kalij pheasant etc in hills of Mizoram, India (Sawmliana, 2013).

No two trees are equal. The adaptive potential of every tree is responsible for the change in character, which in turn is supplemented by a difference in genotypes, environments and their interactions (Savolainen et al., 2011). Varying characters in morphology, phenology and reproductive traits follow wide geographical distribution of P. timoriana. Hopkins (1994) reported that *P. timoriana* thrives best in regions with an altitudinal range from 0 to 600 m and further cited few instances of it growing at 1300 msl in NEI. However, Meitei and Jayalakshmi (2005) reported its presence in different agro-climatic range in Manipur, starting from hotter plains to colder mountain up to 2000 msl. The difference between the two reports may be due to adaptive effect of the species to the changing climate in the region. It was also found that characters such as shape of the capitulum, length of pod, shape of pod, flowering time and fruiting time varied with the change in site Hopkins (1994). Works on the effects of maternal seeds during development by Gutterman (2000) gave a detailed report on the role of environment in seed germinability. The population ecology of *P. timoriana* has not been studied much in the past. From the few works, Singh (2002) studied eight cultivars of *P.*

roxburghii collected from four different places of Manipur. He analysed morphological characters of these cultivars and grouped zimmermanhem into four clusters. However, based on storage proteins and randomly amplified polymorphic DNA (RAPD) analysis, the cultivars were reported to be of the same genotype. This difference in characters maybe because of the maternal environment surrounding them. On the other hand, Thangjam (2016) work on the genetic divergence of this species in three populations in Manipur by using RAPD analysis and Inter-simple sequence repeats marker analysis showed the variations to be mostly genetic. This contrasting result makes it important for further work on population ecology of this species to a wider range and with more number of provenance samples.

Provenance trial focuses on selecting the best performing provenance by comparing its characters, or it can also be used for selecting the best characters by comparing the provenance. Both of which could be used in tree improvement programme. Many scientists have worked on provenance trial related to food crops but few have worked on tree species. Genetic and environmental influences on qualitative and quantitative traits are different for both between species and within species. Work on *Cordial africana* (Loha et al., 2006) reported germinability and seedling height to be more influenced by genetic factors while work on *Dalbergia sissoo* (Singh and Bhatt, 2008) reported it to be influenced by the environment. Therefore, it is important to determine the role of both genetic and environmental factors responsible for a particular trait.

With variation in biophysical and topography of *P. timoriana* distribution, there is a need to develop an agro-climatic map for the selected provenances. By knowing the moisture index and analysing the variance in the reproductive traits of the provenances, it is possible to generate agro-climatic seed map that could predict the possible seed, pod and seedling character. Furthermore, the wide distribution and dominance of *P. timoriana* in home gardens of NEI signify that the species also represent a potential carbon sink in this region. Few works have been done related to tree volume, biomass and carbon stock in NEI (Brahma et al., 2017; Nath et al., 2019) but no work is reported so far for this tree species.

Reproductive traits such as seed germination and seedling growth are affected by its readiness to germinate (dormancy) and the amount of food reserve present (seed weight) in it. Every grower wants his or her seed to germinate fast, uniformly and more of percent germination. However, some seeds fail to meet the above requirement due to unfavourable condition. In natural condition, dormancy is a way to escape unfavourable condition and germinate when the condition subside (Carvalho and Nakagawa, 2000). However, in artificial regeneration growers can't wait for a natural favourable condition but instead have to provide the condition artificially. Pre-germination treatments are ways to enhance germination and also to find the most probable factor causing seed dormancy. *P. timoriana* has a hard seed coat and thus, the seeds are expected to have dormancy of some kind. In addition, the seeds of this species differ in weight and thus good germination from heavier seed is expected due to the presence of more amount of food reserve.

The above study will help us understand the degree in which a particular character of seed, pod, germination and seedling growth are controlled by genetics or environment. It will also give the best performing provenance for all these traits. Agro-climatic map developed may be useful for prediction of seed source characters by knowing the moisture index of an area. Best performed agro-climatic zone for all the characters may be referred for future seed transfer. In addition by finding the best volume model, it could be used further for determining carbon stock and carbon sequestration potential. Study on seed pre-treatment for this species is also important as it will give the most appropriate treatment method to increase germination and reduce germination time. Determining the effect of seed weight on germination and early growth is essential as every plantation requires healthy seedling that has a promise of healthy growth. Thus, the present study encompasses the following objectives:

1) Effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana*.
2) Effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana*
3) Effect of seed source and seed size/mass on seed germination and seedling growth.
4) Seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* in North-east India
5) Developing stem volume equation for *Parkia timoriana* (DC.) Merr.

CHAPTER 2

Review of literature

Provenance variation

Effect of seed source variation and provenance trials are a common area of research for herbs and shrubs but are rare for trees. So far, out of more than 1800 flowering plants reported growing in India less than 20 tree species were studied for variations and include none from northeastern states of India.

P. timoriana is widely grown in home gardens and shifting agriculture lands of North East India (NEI). Morphometric characters of this species vary along with its natural distribution and among cultivated trees. Throughout most of its range, the pods (fruit) are flat and strap-shaped, not corrugated over the seeds and the suture lines are not thickened. The ripe pods rattle. However, in some specimens from NEI and Bangladesh, the valves are somewhat corrugated over the seeds, and in this respect, they resemble *P. leiophylla*. In Asia-Pacific region, the flower heads are usually oblong-pyriform with an ellipsoid apical part and short staminodial flowers. However, those trees cultivated at the Botanical Gardens in Victoria, Seychelles, are clavate, with a spherical apical part, a very constricted nectar-secreting region, and again, little staminodial development (Hopkins, 1994). This cultivated form also has twisted pods, though they are not swollen over the seeds. Provenance variation in flowering and fruiting time was also reported for this tree species. In Singapore *P. timoriana* was reported to bear flowers from September to October, and the fruits ripen at the time of leaf renewal in February (Holttum, 1931,1940), however in India flowering time was reported to occur in June-July and fruit maturation in March-April (Angami et al., 2018).

Many workers also reported differences in morphometric characters in different species which they conclude to be the result of genetic and environmental factors. They also showed a significant correlation between maternal environment, germinability and seedling growth of the seeds after the seeds break dormancy. Maternal factors that were studied under this are: position of seeds or inflorescence on mother plants (Datta et al., 1970; Gutterman, 1996), age of the mother plant (Kigel et al., 1979; Gutterman, 1978); day length (Gutterman, 1996); temperature (Ghosh and Singh, 2011); rainfall (Eleanor et al. 2007); light quality (Khan, 2004); and altitude (Singh et al., 2006).

Parkia timoriana though has reported on variations in some of its traits in different regions, has a lack of deeper scientific research on the individual traits representing individual characters for heredity and regeneration. Moreover, no studies have been made so far on the relation between provenance, maternal environment and germinability and seedling growth of the species.

Role of seed pre-treatments

Seed germination starts with the uptake of water by the quiescent dry seed, ending up with the elongation of the embryonic axis (Holdsworth et al., 2008). In many plant species, seed maturation and germination are separated by a period of low metabolic activity referred to as quiescence or dormancy. Dormancy has evolved differently across species through adaptation to the prevailing environment, to allow seeds to germinate only when conditions are likely to favour the establishment of a new plant (Baskin and Baskin, 2004). Seed dormancy can be defined as the failure of seeds to germinate owing to factors associated with their seed coat or embryo. It may be due to the seed coat being impermeable to water or oxygen or both, hard seed coat, immature embryo, embryos that require an after-ripening period or maybe as a result of endogenous chemical germination inhibitors (Wareing and Phillips, 1981). The degree of dormancy makes it difficult for the seed to germinate evenly and adequately. Slow emergence results in smaller plants and seedlings, which become more vulnerable to soil-borne diseases. Studies carried out on several species for pre-germination treatments of seeds (Thakur and Sharma, 2005; Farooq et al., 2005; Basra et al., 2007) showed that different species call for different pre-treatments for breaking seed dormancy.

P. timoriana seeds have a hard seed coat hence their propagation may be adversely affected by seed coat dormancy resulting in poor imbibitions and germination potential (Bradbeer, 1988). The causes of seed coat impermeability in the species are not fully understood, but it has been observed that under natural conditions and after most treatments the first site at which water penetrates occurs is the stophioles (Harper, 1977). This is the weakest and the reinforced area of the side opposite the micropile. *P. timoriana* seeds need to be subjected to some physical or chemical treatment to break dormancy to obtain uniform germination. Various works have employed different methods of breaking or reducing dormancy and improving germination rate in different forest seeds including cold water (Eze and Orole, 1987), hot water (Otegbeye and Momodu, 2002; Oni et al., 2005) and acid treatment (Ibrahim and Otegbeye, 2004; Dachung and Verinumbe, 2006; Isikhuemen and Kalu, 2006). Time of immersing in the acid is critical since long soaking periods can excessively burn the seed coat and damage the embryo (Schmidt, 2000). Mechanical scarification is also known to break physical dormancy of hard-coated seeds which inhibit water uptake and gases such as in *Acacia* species (Doran et al., 1983; Tietema et al., 1992; Hossain et al., 2005). These treatments according to Umar (2005) are aimed at making the seed coat permeable either naturally or artificially and hence enhances germination.

Besides, inadequate information about seed pre-germination treatments causes lack of assurance on rapid and uniform germination of better quality seedling of *P. timoriana*.

Further, the need to better understand the reproductive cycle of this species arises as few previous studies reported relatively low germination percentage in this species.

Role of seed weight

Numerous ecological and evolutionary factors can affect seed germination process and further plant establishment (Marques and Oliveira, 2008). Seed mass has been considered as an important evolutionary trait that affects the reproductive outcome of many plant species (Cordazzo, 2002). It has a direct influence on the germination percentage (Mölken et al., 2005), mean germination time (Murali, 1997), and seedling vigor (Yanlong et al., 2007), which in turn may indirectly influence plant distribution and abundance across different habitats (Westoby et al., 1992). However, seed mass-produced by plants varies both between and within plant species (Leishman et al.,1995; Silvertown and Bullock, 2003; Moles and Westoby, 2006). Differences in seed mass have been related to the ecological conditions in which plant establish, with species from close habitats having higher seed mass than species from more opened habitats (Foster and Janson, 1985).

Germination, growth and survival of seedlings are influenced largely by the food reserve in seeds (Tripathi and Khan, 1990; Khan and Uma Shankar, 2001). Foster (1986) argued that large seed reserves might be used for the construction of a large amount of photosynthetic tissue in order to maintain a positive net energy balance or might also allow quick seedling growth for reaching higher light intensity strata. Large and heavy seeded species have an advantage in competitive environments (Gross, 1984) and when seedlings experience moisture stress (Armstrong and Westoby, 1993) or defoliation (Baker, 1972). Generally, within species, heavier seeds tend to give better germination than lighter ones. However, many reports of conflicting results also exist and cannot be neglected. For instance, large seeds may germinate with higher percentages than small seeds (Tripathi and Khan, 1990; Bhuyan et al., 1996; Khan and Uma Shankar, 2001) and small seeds may germinate with higher percentages than large seeds (Marshall, 1986), or germination may be independent of seed size (Gross and Kromer, 1986; Perez-Garcia et al., 1995). Within a species, heavier seeds may take less time for germination than lighter seeds (Barik et al., 1996); lighter seeds may germinate earlier than the heavier seeds (Murali 1997; Khan et al., 1999), and germination time may be independent of seed weight (Perez-Garcia et al., 1995).

Heavy seeds tend to produce more vigorous seedlings when compared to lighter seeds (Yanlong et al., 2007). However, plants respond to their environment in such a way as to optimize their resource use. Thus, according to the resource optimization hypothesis, plants allocate relatively less resource to their root system when nutrient availability increases (Agren and Franklin, 2003). Therefore, it is expected that seedlings originated from larger seeds would present a smaller root: shoot ratio, as large seeds have more nutritional reserves.

Another important determinant of initial seedling growth is seedling relative growth rate (RGR). Many workers have reported a negative relationship between seed mass and RGR, in particular across species (Reich et al., 1998, 2003; Wright and Westoby, 1999; Grotkopp et al., 2002; Poorter and Rose, 2005). A negative seed mass-RGR relationship may be a determinant of plant demography and community composition because it could allow small-seeded species to compensate for their lower seed reserves and thereby overcome the initial advantage conferred to species with grater seed masses (Norgren, 1996; Paz and Martinez-Ramos, 2003). However, this explanation could apply within species too, so that individuals derived from small seeds could compensate for their small seed reserves by a higher RGR (Zimmerman and Weis, 1983). The relationship between seed mass and RGR within species has rarely been analysed and no report for *P. timoriana*. Few within species reports available offer contrasting results, with either a negative correlation between seed mass and RGR (Meyer and Carlson, 2001; Paz and Martinez-Ramos, 2003), a lack of correlation (Tamet et al., 1996) or a positive correlation (Meerts and Gernier, 1996), thus contradicting general trends in cross-species comparison.

Variation of seed size is an important area of plant ecology because seed size can directly affect the process of germination and seedling recruitment, influencing the plant performance under different environment (Leishman, 2001; Rego et al., 2007). In NEI, *P. timoriana* represents wide geographic distribution, occurring at different agroclimatic zones. Hence, it is important to determine the relationship between seed mass with germination time, germination percentage, seedling vigour, seedling root/shoot ratio and RGR.

Agro-climatic zonation and Thornthwaite model

Climate classifications are methods used to identify climate types (Gallardo et al., 2013; Jacobeit, 2010) and spatial and seasonal climate variability (Bieniek et al., 2012). There are several climate classification systems (CCSs), such as Holdridge (1967), Flohn (1950), Camargo (1991), Köppen and Geiger (1928) and Thornthwaite (1948). The latter two are the most used worldwide (Spinoni et al., 2014). CCSs are used in the validation of climate models (Jylhä et al., 2010; Belda et al., 2014), in climate change studies (Mahlstein et al., 2013) and in the definition of agro-climatic zones (Rahimi et al., 2013). The CCs proposed by Thornthwaite (1948) (TH), in addition to air temperature and precipitation, uses potential evapotranspiration together with the elements of water balance to analyse climatic zones (Elguindi et al., 2014), defining a climate as dry or humid relative to the water needs of crops (Feddema et al., 2005). In the Thornthwaite (TH) CCS, the tree is viewed as the physical medium by which water is transported from the soil to the atmosphere. Thus, a type of climate is defined by TH as humid based on the water needs of the plants.

Few works have been done on the effect of established agro-climate on the seed characters. However, no work has been done yet to find out the agro-climate of a seed source using standard model, followed by analysing the difference in regeneration and growth characters between agro-climates, and to give an overall map of the different agro-climate using GIS tools.

Volume equation development

The importance of volume in forestry could be traced back to the starting of human civilization. Archimedes, the pioneer of fluid dynamics, gave the first written equation for volume estimation two thousand years ago and since then many scientists keep on adding other new equations and functions for different applications. Volume and volume equation plays a pivotal role in silviculture, forest mensuration, management and in the estimation of carbon stocks and carbon sequestration potential of trees. Every tree has a particular structure that can be represented by its diameter, height and form. Hence, a particular volume equation may be a good fit for one species but not to another. On the other hand, the same species in different geographical areas may give slightly different increments, tree form and branching pattern. Therefore, there is a need for constant upgrading and/or development of new volume equation that fits diverse geographical range. For example, work done on *Dalbergia sissoo* (Khan and Faruque, 2010), *Pinus* spp (Lee et al., 2014, 2017), and *Pinus thunberii* (Park et al., 2015) have shown constant improvement on the already established equations while work on *Aquilaria malaccensis* (Islam and Chowdhury, 2017) and *Eucaluptus* hybrid (Tewari and Singh, 2006) developed new volume equation. Nevertheless, a critical review on model development, common mistakes and corrective measures have been discussed and use of fewer explanatory variables has been recommended for ease model application and validation (Sileshi, 2014, 2015).

In India, Forest Survey of India (FSI) has been working to generate volume (growing stock) of various trees since 1965 when it was first called Pre-investment Survey of Forest Resources (PISFR). Since then various workers have developed volume equations for different fast-growing and economically important trees in different parts of the country. Volume estimation of trees can be performed in two different ways, one by felling the tree (destructive method) and the other by non-contact measurement (non-destructive method) wherein, already established species-specific models or techniques and instruments are used. Destructive method of sampling involves felling the tree, cutting down to the smallest diameter, measuring the length and basal area and finally estimating the volume. Although it gives the most reliable estimates of volume, it is not always feasible as this method is costly, time-consuming and most importantly destructive. Moreover, some species having high ethnobotanical and economic values are difficult to fell, therefore the need for an alternative method of volume determination becomes a necessity. There are many ways of non-destructive volume estimation, however the most common among all is the derivation of tree volume from species-specific volume equations given by FSI (Forest Survey of India) or from other individual research findings. These established equations are region-specific and yet the best fit model of many tree species are to be determined. Other important non-destructive methods include; photogrammetry (Berveglieri et al., 2017; Mokroš et al., 2018), 3-D laser scanning technology (Liang et al., 2018; Astrup et al., 2014), UAV technology (Wieser et al., 2017), optical-electronic total station (Yan et al., 2012; Feng et al., 2017), etc. These recent technologies and techniques are reliable but because of its non-portability and management cost issues, few are applicable in practical forestry study. Furthermore, the hardware of Photogrammetry, 3-D laser scanning technology and UAV (Liang et al., 2018; Wieser et al., 2017; Mikita et al., 2016; Liu et al., 2016; Fankhauser et al., 2018) and the compiled data is difficult to process, primarily because point cloud data requires high-end computer and costly software. On the other hand, the optical-electronic total station method is very cumbersome and very complicated to operate in forestry surveys (Yan et al., 2012; Feng et al., 2017). Few scientists have used low-cost technologies such as electronic theodolite in forest surveys; however, the sole use of this instrument for measuring whole tree volume was not very accurate. Therefore, standing tree volume calculation requires a new non-destructive, pocket-friendly and highly efficient methodology using locally available instruments. Our present non-destructive method makes use of locally made 30° angle scale, 60x zoom Nikon digital camera, and a measuring tape. The procedure is similar to Montes method (Montes et al., 2000), however instead of scanning the photograph and making grids we used trigonometric principles to calculate the branch diameter.

P. timoriana is mainly concentrated in parts of eastern Himalayas of India though found scattered in other South-East Asian countries too. It served as an important food supplement with varied uses which led to its dominance in home gardens and shifting agriculture lands of this region (Sahoo, 2009). Shifting cultivation (locally known as 'jhum') is a type of traditional land use system in which a vast area of land is burnt, cultivated for 2 to 3 years and then shifted to another patch of land and repeats the same process. In spite of immense economic importance, this tree has not been systematically evaluated mostly because of insufficient knowledge on volume and biomass. This may be because the destructive method of estimating volume is difficult for this species as most of the stakeholder of *Parkia* trees are local tribal whose livelihood depends on the income generated by selling the fruits (pods). Hence, experimental set up for volume estimation for this species could only be performed through allometric and non-destructive means, which consequently make our study important.

CHAPTER 3

Species description, distribution and study sites

Introduction

Parkia timoriana is a genus of the tribe Mimosae (family Fabaceae, sub-family Mimosoideae) with 31 species. It was named in memory of celebrated Scottish explorer, Mungo Park (Hopkins, 1994). The synonyms of *P. timoriana* are *Parkia roxburghii* G. Don, *Acacia niopo* Litv., *Inga timoriana* DC., *Mimosa peregrina* Blanco, *Parkia calcarata* Lecomte and *Parkia grandis* Hassk (Angami et al., 2018). It is a very important multipurpose tree producing unique flower and fruit that support the livelihood of people in its distribution range.

Vernacular names

The plant has been locally recognized with different vernacular names in India (Firake et al., 2013) and other countries (Hopkins, 1994). Vernacular names given to this species in India are: Yongchak, Uroi (Manipuri); Zawngtrah (Mizo); Unkamn-pinching (Naga); Aoelgap (Garo); Khorial (Assamese); Bire-phang (Kachari); Manipuri seem (Bengali); Themuk-arang (Mikir); Shivalingada mara (Kannada); Unkampinching (Marathi). Vernacular names given in other countries include: Mai-Karien (Shan, Burma); Riang, Karieng (Thai, Thailand); Kedayong, Petai (Malaya Peninsula); Alai (Indonesian, Sumatra); Kedawung, Peundeuj, Dawung, Petir (Java); Kopang (Indonesian, Sumbawa); Koepang (Bandji, Kalimantan); Buah batar (Kelabit, Sarawak); Timbarayong (Sabah); Olimbopo (Tolalaki, Sulawesi); Amarang (Palawan); Cupang or Kupang (Taf, Luzon). Approved vernacular name in timber trade for *P. timoriana* is *petai kerayong*.

Phenology

Parkia timoriana is a large tree (up to 25-30 m height) with spreading branches (Curtin and Chivers, 1987). It shows a regular annual phenological cycle with a brief period of leaflessness followed by a flush of new leaves; widely dispersed individuals flush synchronously (Hopkins, 1994). Flowering and fruiting occur in most years (Saw et al., 1991). Flowering starts by last week of July and takes about four months from anthesis to fruit ripening. Anthesis is nocturnal, and each capitulum functions for a single night, the flowers opening gradually during the preceding day. The leaves are bipinnate with numerous small curved leaflets and flowers are dense turbinate or clavate heads hanging on long peduncles (Angami et al., 2018). At the age of 6 years, the plant starts its production; however, the full bearing stage is only after 10 years. The life span of this tree may range from 80-90 years or more.

Floral biology and pollination

The inflorescence is head or capitulum of racemose type with clusters of yellowish-white tiny flowers, hanging at top of long stalks from the branches and emerges during June-July. The capitula are composed of three different morphological and functional sorts of flowers, though there is some gradation between them (Hopkins, 1986). The fertile flowers form a ball at the apex. Below them is a ring or band of shorter, thickened, nectar-secreting flowers that usually lack a functional gynoecium. At the base, there is a ring of staminoidal flowers that are morphologically male but functionally neuter. The fertile flower has five calyx lobes which are partially gamosepalous and petaloid. Corolla lobes are five, membranous, polypetalous which are attached to the tubular structure of stamens about 2-2.5 mm above the calyx. There are 10 whitish stamens fused one another at the base and forms a tubular structure called staminal tube. The ovary is simple with a single stigma and has marginal placentation (Hopkins, 1994). Flowering starts by last week of July and fruit set starts after 10-15 days of flowering.

The young inflorescences are protected by foliaceous bracts which are free from each other. At the base of every flower, there is a thin membranous structure called bracteole; it covers individual flowers in inflorescence during juvenile stages thereby imparting its brown greenish colour to the head (Hopkins, 1994). The heads produce numerous flowers, the majority of which are fades and drops off.

Wood anatomy

The wood of *Parkia* is classified as light hardwood, soft to moderately hard and light to moderately heavy (Hopkins, 1983). Anatomical characters showed growth rings as indistinct, radial multiples as 2-5 per mm^2, vessels of 3-9 per mm^2, inter vessel pits (IVP) diameter of 6-10 μm, number of cells per stand of 1-5, 5-18 homogenous rays per mm, absence of septate fibres and with marginal, aliform, confluent broad, broad-banded axial, confluent marginal, vascicentric, unilateral, occasionally axial/ confluent parenchyma strand (Mundotiya et al., 2016).

Seed biology

Pods are produced in bunches of up to about 15, each measuring 25-40 cm in length and 2-4 cm in breadth and pendent from a single swollen receptacle. These pods in early stages are soft, tender and light green in colour, which on maturation turns black, and contain yellow dry powdery pulp with several seeds embedded in it (Longvah and Deosthale, 1998). The testa of the seed is hard and dark at maturity that took over a period of weeks following planting. The number of seeds per pod depends on the dimension of the seeds and pods and also on the location or source of the seeds.

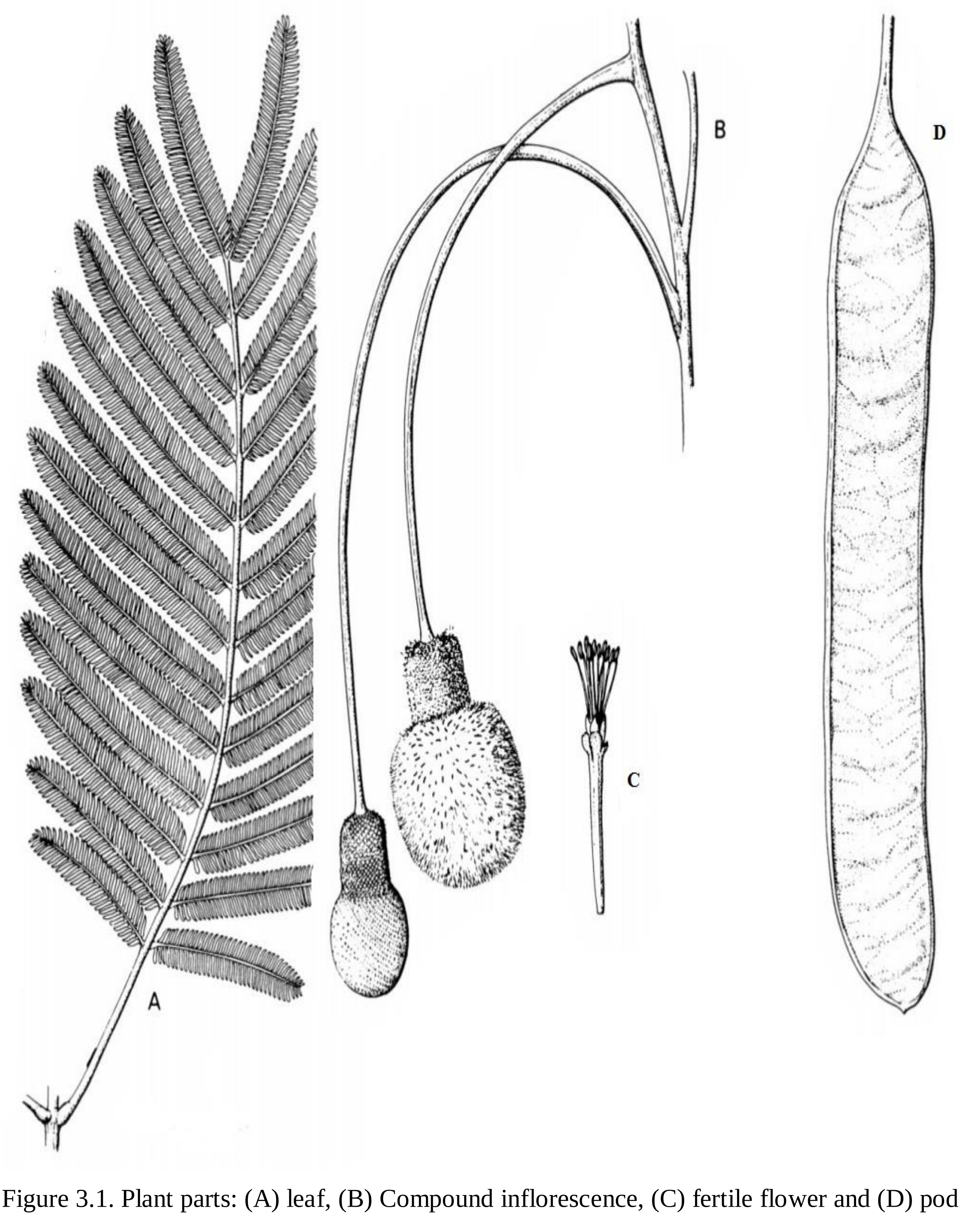

Figure 3.1. Plant parts: (A) leaf, (B) Compound inflorescence, (C) fertile flower and (D) pod

Distribution

Parkia timoriana (DC) Merr. (Family: Mimosaceae) is one of the well known multipurpose tree species found in of tropics and subtropics, and has a wide distribution in North East India and other South-East Asian countries like Indonesia, Japan, Malaysia, Philippines, Thailand, Vietnam and Northeast India (Salam et al., 1998). This is the only species of *Parkia*, which is found in both sides of Wallace's line, primarily distributed in the evergreen rain forest, moist mixed deciduous and dry evergreen forests. Altitudinal variation of this species usually range between 0 to 600 m, most common up to 300 m. Maximum height was reported from the upper limit of a dipterocarp forest in Borneo, however in North East India and Bangladesh it rarely reaches 1300 m (Hopkins, 1994).

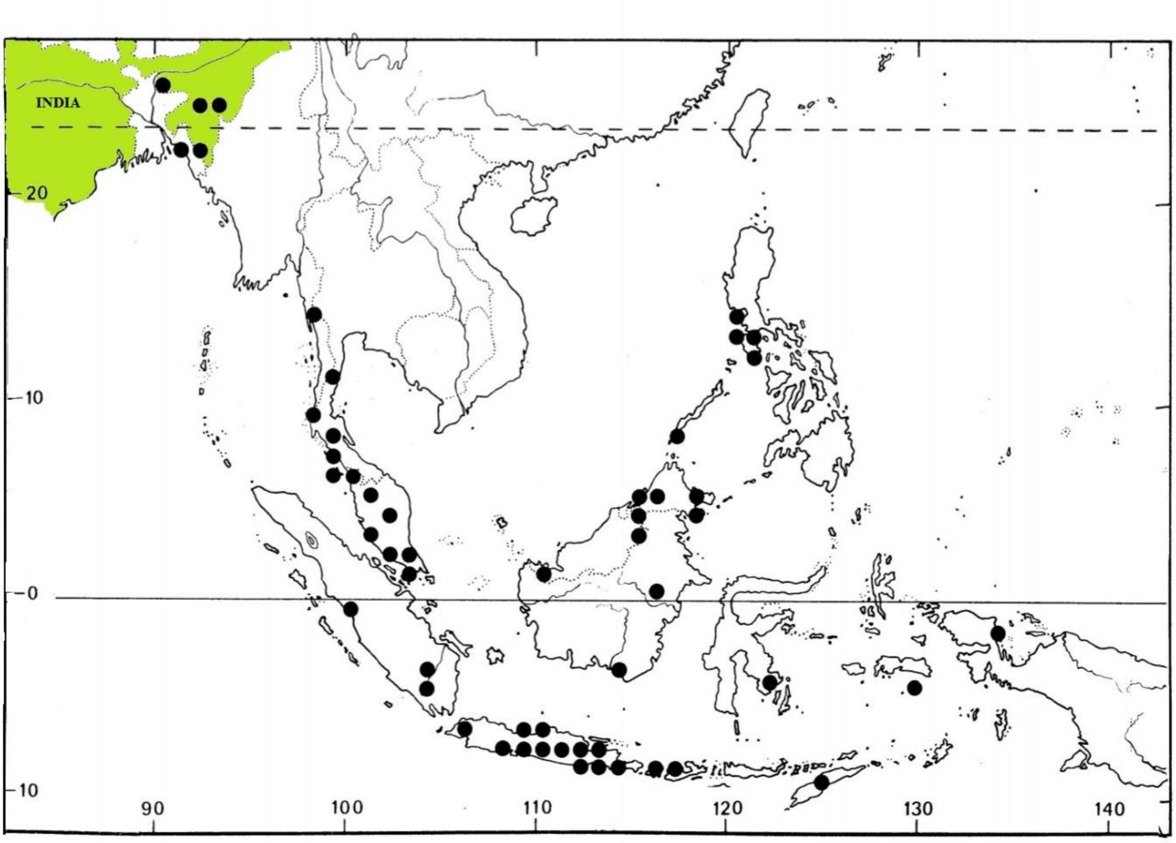

Figure 3.2. Asia-pacific distribution of *Parkia timoriana*

Study sites

Provenance represents the original native place where the seeds are sourced (Zobel and Talbert, 1984). For finding the effect of provenance variation on morphometric, reproductive and seedling traits, and for defining agro-climatic zones using Thornthwaite model, 12 provenances representing 4 north-eastern states of India were selected based on an extensive survey. The 12 provenances were; Pherema and Medziphema in Nagaland, Shillong and Sumer in Meghalaya, Bishnupur, Senapati, Jiribam, Langol and Achanbigei in Manipur, and Serchhip, Lunglei and Sakawrtuichhun in Mizoram. These sites represent wide variation in both geographic and climatic variables. Minimum altitude among the study sites was associated with Jiribam provenance (60 m) and maximum height with Shillong provenance (1428.5m). Likewise, rainfall and temperature also vary significantly. This study did not found the population of this species in wild. Therefore, all the samples were taken from home gardens and shifting cultivation areas. Works carried out in some of the home gardens in these regions found *P. timoriana* as one among the dominant species (Devi and Das, 2012; Jeeceelee and Sahoo, 2015).

Furthermore, to determine the best fit volume model of *P. timoriana* tree by non-destructive method, a 13th population (Tanhril, Mizoram) was taken. The resultant best model was cross-validated with this extra population to see the corroboration. Finally, the remaining two objectives on the effect of pre-treatments and seed weight on germination and growth use seeds from a single seed source. Seeds from Lunglei provenance were used for pre-treatment while seeds from Sakawrtuichhun provenance were taken for weight experiment. All the provenances are represented in figure 3.3.

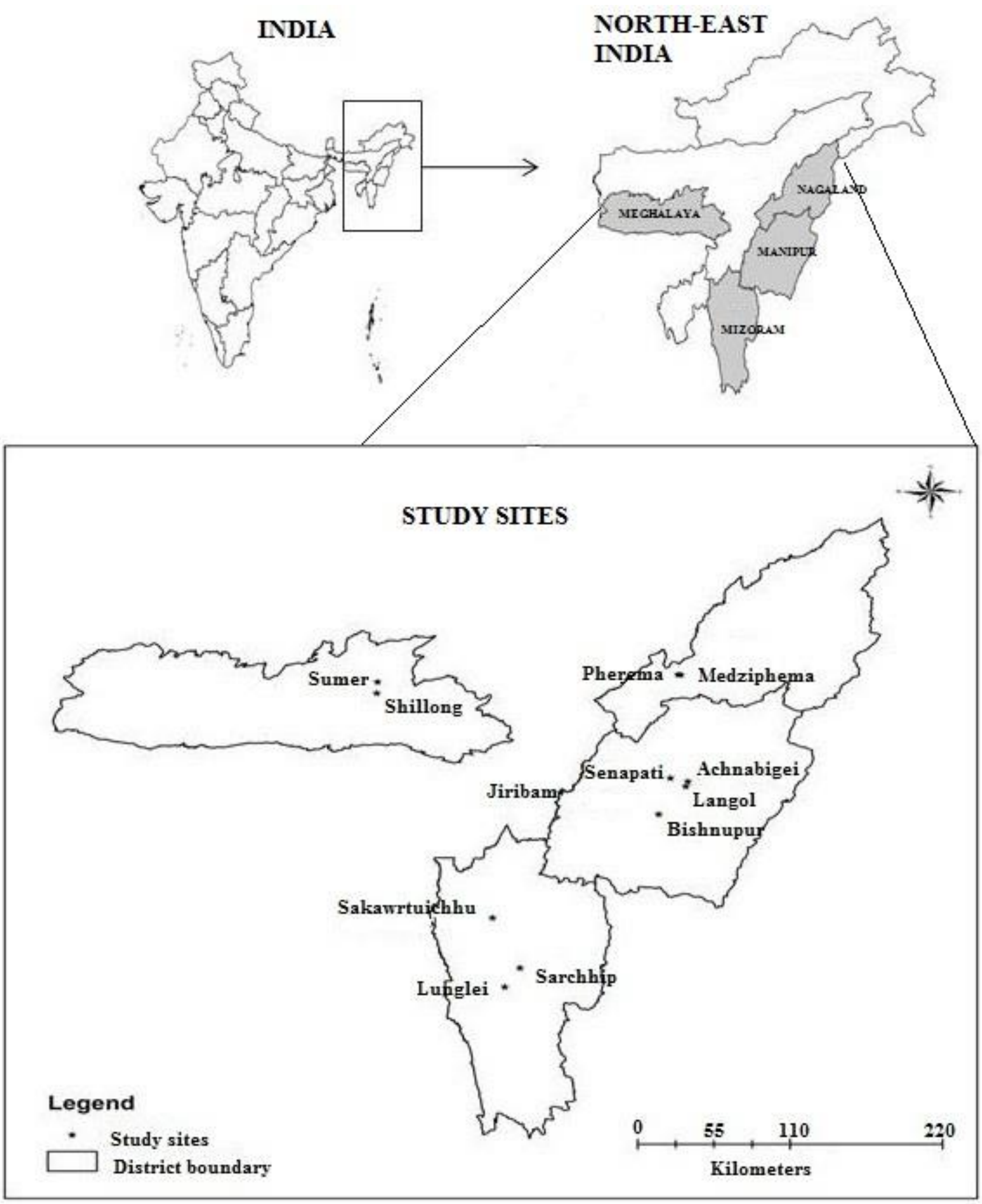

Figure 3.3. Map of India showing 12 provenance of *Parkia timoriana* in Northeast India

Soil physicochemical properties

Provenance trial includes the selection of wide geographical distribution of species. Therefore, the presence of different physicochemical properties of soil between the sites is expected and needs verification. Three random points were selected from each provenance, and from each point, two soil samples were collected: one from surface (0-10 cm depth), and another from sub-surface (10-20 cm depth). These soil samples were then transferred from land to lab and various physicochemical properties were analysed. Soil pH for each sample was measured by transferring 10 g of fresh soil in 100 ml beaker containing 50 ml of distilled water. The above solution was then mixed for 20 minutes by using a magnetic stirrer. The soil-water mixture was kept overnight and then pH was recorded with the help of digital pH meter. Soil texture was determined by Bouyoucos hydrometer method (Allen et al., 1974). Soil organic carbon (SOC) was determined by rapid dichromate oxidation technique (Walkley and Black, 1934). Here, the organic matters in the soil were oxidized by chromic acid (Potassium dichromate plus conc. Sulphuric acid) utilizing the heat of dilution of sulphuric acid. The unreacted dichromate was determined by back titration with ferrous sulphate. Available nitrogen was determined by alkaline permanganate method (Subbiah and Asija, 1956) and available phosphorus by Bray and Kurtz (1945) method. Finally, available potassium was measured by using Hanway and Heidel (1952) method. The soil physicochemical properties of the provenances are shown in table 3.1.

Table 3.1. Physico-chemical properties of soil of different provenances of *P. timoriana*

Provenance	Soil texture	Depth	pH	SOC(%)	Available N(Kg/ha)	Available P(Kg/ha)	Available K(Kg/ha)
P1	Loam	0-10 cm	4.07±.02	1.01±.04	168±4.7	10.91±.13	143±5
		10-20 cm	4.15±.01	0.86±.01	140±14	10.04±.34	122±3
P2	Sandy clay loam	0-10 cm	4.72±.01	0.41±.03	182±9.3	9.26±.24	251±3
		10-20 cm	4.57±00	0.49±.01	168±00	8.46±.21	204±2
P3	Sandy loam	0-10 cm	5.16±.03	2.48±.05	140±4.7	9.82±.18	50±4
		10-20 cm	5.21±00	2.23±.04	126±4.7	9.05±.3	54±2
P4	Sandy loam	0-10 cm	5.51±.02	2.03±.03	168±00	10.14±.37	49±3
		10-20 cm	5.39±.01	1.87±.02	154±4.7	9.42±.12	74±5
P5	Sandy clay loam	0-10 cm	6.18±00	2.06±.01	238±14	11.48±.45	450±15
		10-20 cm	6.05±00	1.88±.03	196±00	10.69±.16	412±8
P6	Sandy loam	0-10 cm	4.46±00	1.13±.03	126±14	9.52±.32	348±6
		10-20 cm	4.51±.02	1.05±.03	98±9.3	9.45±.06	318±11
P7	Loam	0-10 cm	5.72±.01	0.14±.03	126±4.3	7.83±.21	266±14
		10-20 cm	5.78±.04	0.09±.01	98±9.3	7.54±.47	279±16
P8	Sandy loam	0-10 cm	6.57±00	0.83±.06	146±4.3	8.87±.04	531±11
		10-20 cm	6.21±.02	0.75±.04	112±4.7	8.03±.52	404±5
P9	Loam	0-10 cm	5.59±.02	0.49±.05	308±4.7	11.59±.18	57±7
		10-20 cm	6.13±00	0.38±.01	266±00	11.19±.18	60±4
P10	Sandy loam	0-10 cm	5.32±00	0.98±.05	168±00	9.24±.21	101±5
		10-20 cm	4.79±00	0.83±.02	182±00	8.88±.26	86±5
P11	Sandy loam	0-10 cm	4.64±.08	1.05±.02	252±9.3	7.97±.09	189±9
		10-20 cm	4.36±.03	0.87±.02	266±4.7	7.13±.15	121±14
P12	Loam	0-10 cm	4.49±00	0.45±.03	266±00	9.1±.31	209±12
		10-20 cm	4.21±.03	0.41±.04	280±9.3	8.35±.19	186±8

±SEm, n=3

Uses

The plant provides a good source of livelihood for both hill and valley people during winter to the early spring season. Pods are sold at high price fetching a market value ranging from ₹ 70 to 120 Kg^{-1} (Firake et al., 2013). They serve as an important seasonal food and also a good source of protein (Mohan and Janardhan, 1993) and antioxidant (Tapan, 2011); bark, seed and pod are used as a good and effective medicine for curing various ailments (Angami et al., 2018), pod and bark extract is applied on skin as a natural cosmetic (Bhuyan, 1996) while branch and stem are used as firewood (Rocky and Sahoo, 2004) among the tribes of India.

Insect and pest attack

P. timoriana tree is vulnerable to the pest insect *Cadra cautella*, a moth whose larva bores into the seed to pupate, feeding on the seed interior and filling it with webbing (Thangjam et al., 2003). *Parkia biglobosa*, another species of this genus in Africa have shown lack of regeneration and ageing of the stands, which might result in complete disappearance over time (Nikiema, 1993; Quedraogo, 1995; Bouda and Nikiema, 1996; Ræbild et al., 2012). Over-exploitation, insect infestation, shortening of fallow period in shifting land use and drier climate could cause the complete disappearance of this species unless immediate measures are undertaken (Nikiema, 1993; Teklehaimanot, 2004; Roy, 2016).

CHAPTER 4

Effect of provenance on morphometric, reproductive and seedling traits of *Parkia timoriana* (DC.) Merr.

4.1 Introduction

Parkia timoriana (DC.) Merr., commonly known as Tree Bean (www.theplantlist.org), is one of the most widely distributed species of *Parkia* found in both sides of the hemisphere (Indo-Pacific region) and the only species found in both sides of the Wallace line (Hopkins, 1994). This genus includes 31 species which was first documented by R. Brown (Hopkins, 1986) who distinguished it from other mimosoid genera by the imbricate aestivation of the unequal calyx lobes. Six species namely: *Parkia timoriana* (DC.) Merr., *P. biglandulosa* Wight et Arn., *P. speciosa* Hassk., *P. filicoidea* Oliv., *P. bicolour* A. Chev. and *P. clappertoniana* Keay, are reported to present in India (Angami et al., 2018). Morphologically *P. timoriana* is very similar to *P. speciosa* and *P. biglandulosa*, however, it can be distinguished from the former by the absence of pubescence in the outer corolla lobes and by the acute tip of leaflets, and to the later by its broader, non-linear and sigmoid leaflets (Hopkins, 1994). In India, the tree grows luxuriantly in wild, in home gardens and in shifting agriculture lands in the North-Eastern states, particularly Manipur, Mizoram, Nagaland, Meghalaya and Assam (Kanjilal et al., 1982). The plant provides a good source of livelihood for both hill and valley people during winter and early spring season. Pods and seeds are taken as food and are sold at high price fetching a market value ranging from Rs. 70 to 120 Kg^{-1} (Firake et al., 2013). Other important uses of the tree include antioxidant property, ethnomedicinal and cosmetic uses, and firewood supply for the energy-deficient hill people (Angami et al., 2018). Along with its wide uses, there is an urgent need of research on population and regeneration ecology as report on dying of this tree is increasing year after year. Insect pests such as *Cadra cautella Walker* and other heartwood borer damage heartwood, pod and seeds causing dieback of the tree (Thangjam et al., 2003).

Adaptive potential of every tree is responsible for the change in character which in turn is supplemented by a difference in genotypes, environments and their interactions (Savolainen et al., 2011). *P. timoriana* like many other species of Mimosoideae have self-incompatibility. Pollen of this flower could not successfully fertilize with the ovule of the ovary directly without any external help. Hence, large gene flow is expected viz-a-viz genetic variation among the population. Moreover, natural habitat range of *P. timoriana* varies extensively from foothill to elevation up to 1300 m above sea level (Hopkins, 1994) within northeast India and thus, one may expect genetic divergence among its populations in several traits. A study was done by Shao (2015) on *Prima merrilliana* comparing genetic diversity and gene flow in mountainous habitat and foothill showed high genetic variation with unidirectional gene flow from high to low altitude. Few studies have been carried out on the genetic divergence of this species. For instance, Thangjam (2016) studied the genetic divergence of this species in three populations in Manipur by using randomly amplified polymorphic DNA analysis and Inter-simple sequence repeats marker analysis. Molecular markers though provide a powerful means of assessing genetic structuring both within and among populations, many a time they have shown to be poor predictors of variation of adaptive traits (McKay et al., 2005). Therefore, scope of further research involving a wider geographic range of discontinuity with diverse environments and without molecular markers can be of significant importance.

Assuming that variation in phenotypic traits are influenced by local adaptation, maternal environment and varied breeding behaviour, we made an attempt to relate the available variations in phenotypic traits of this species with the geographic variations and seed source, and to identify best seed source to counter the dying population of this species and to develop a general seed transfer guidelines for productivity improvement in the Eastern Himalayan region.

4.2 Materials and Methods

4.2.1 Seed source/ Provenance

Twelve provenances representing 4 north-eastern states of India were identified and selected for the present study for analysing the variation in different quantitative traits. The survey for identifying the provenance was done through three basic steps: (a) through a market survey to find the main source of *Parkia* pods for household consumption and income generation; (b) an extensive field visit to localities where the species is grown abundantly and to cross-check if the source is providing sufficient products to the market and (c) to examine if the species are grown was of the same species by evaluating parameters as per Hopkins (1986). Only mature plus trees of 12±2 years were taken for this experiment to maintain homogeneity in the result. The geo-coordinates and other physical attributes of the population are shown in Table 4.1. The mean seasonal rainfall and temperature data of these populations are shown in Figures 4.1 and 4.2.

Table 4.1. Geomorphological data corresponding to each provenance of *P. timoriana* in Northeast India

Provenance	Code	District	Latitude	Longitude	Altitude (m)	Slope (˚C)	Aspect
Pherema	P1	Dimapur	25˚45′21″N	93˚53′39″E	432	10-15	E
Medziphema	P2	Dimapur	25˚45′30″N	93˚52′30″E	466	5-10	NW
Shillong	P3	Shillong	25˚35′80″N	91˚55′21″E	1428.5	0-5	NE
Sumer	P4	Ri-Bhoi	25˚41′50″N	91˚54′99″E	849.5	10-15	NW
Bishnupur	P5	Bishnupur	24˚36′34″N	93˚45′05″E	820	0-5	SW
Senapati	P6	Senapati	24˚54′25″N	93˚49′36″E	886	0-5	E
Jiribam	P7	Jiribam	24˚47′15″N	93˚06′56″E	60	0-5	N
Langol	P8	Imphal West	24˚50′25″N	93˚55′39″E	851	15-20	SW
Achanbigei	P9	Imphal East	24˚52′46″N	93˚56′25″E	788	5-10	W
Serchhip	P10	Serchhip	23˚20′46″N	92˚51′02″E	858.5	20-25	SW
Lunglei	P11	Lunglei	23˚11′26″N	92˚45′06″E	809	30-35	NW
Sakawrtuichhun	P12	Aizawl	23˚45′33″N	92˚40′25″E	839	10-15	SW

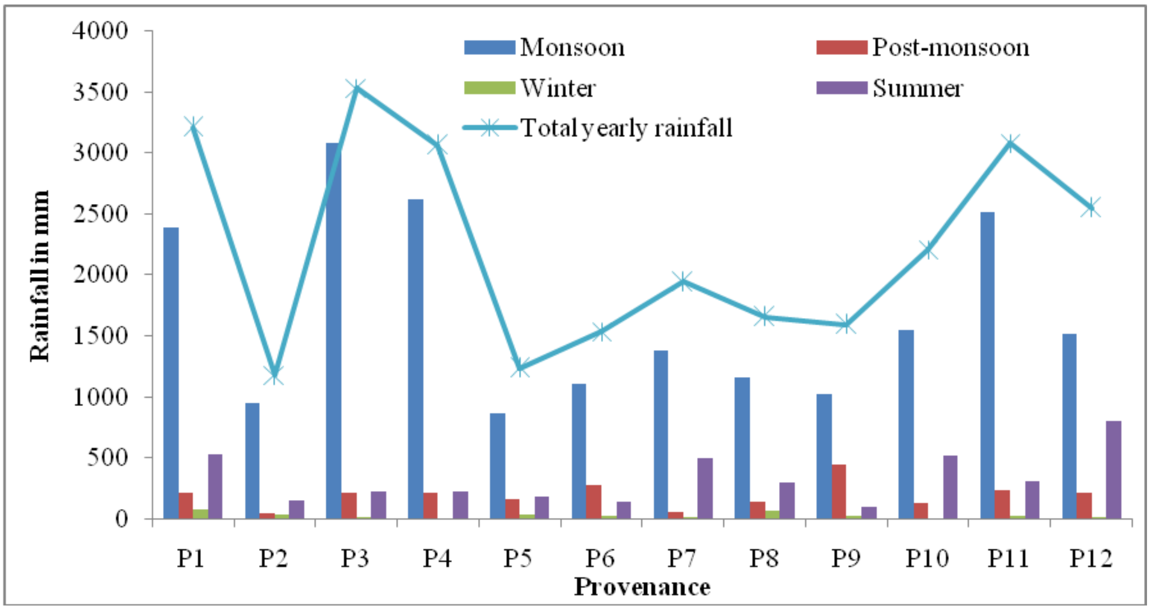

Figure 4.1. Mean seasonal variation in rainfall in different provenances of *P. timoriana* in Northeast India

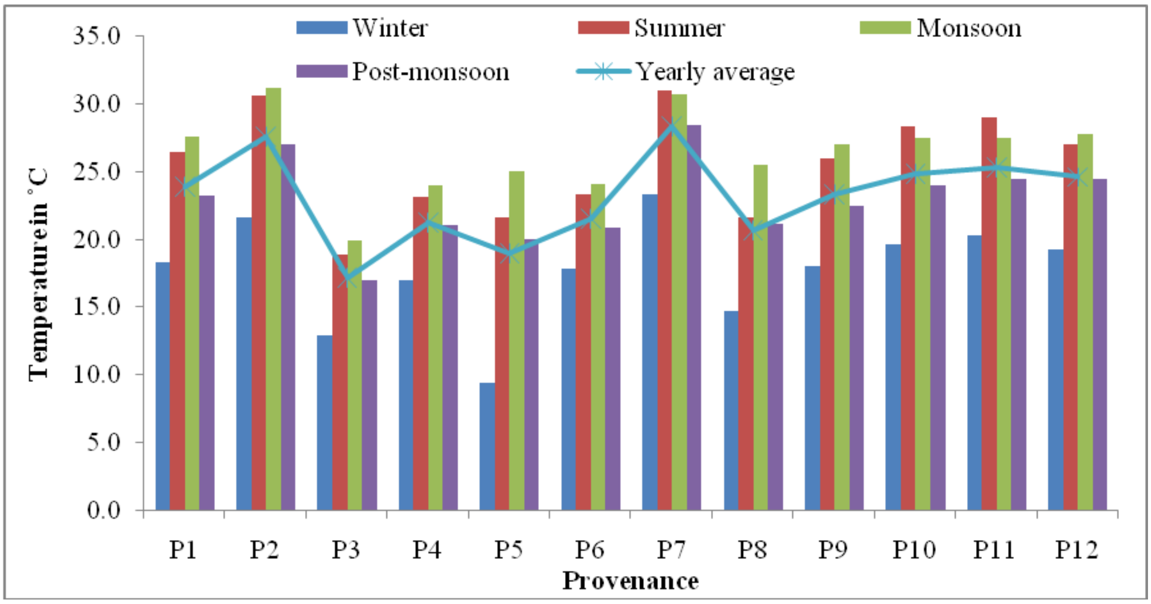

Figure 4.2. Mean seasonal variation in temperature in different provenances of *P. timoriana* in Northeast India

4.2.2 Candidate plus trees selection

The selection of individual superior trees of *P. timoriana* for pod and seed collection was based on the phenotypic assessment of superior characters. In all the 12 provenances, 10 random quadrats per provenance having size 30 x 30 m were assigned at a minimum distance of 10 m apart. Then, 10 most superior candidate plus trees (CPTs), approximately 12±2 years of age, maximum diameter at breast height, maximum height, good seed-bearing history, free from diseases and those which do not have any evident advantage of fertilizer, spacing and cultural practices are eventually selected per provenance by comparing 5 comparison trees per quadrat. Measurements of the five comparison trees were averaged, totalled and then compared with that of the candidate tree. The method of minimum selection standards developed by Pitcher and Dorn (1967) was followed for selection of the plus trees.

Table 4.2**.** Tree characters corresponding to each provenance of *P. timoriana*

Provenance	HT	DBH	CS	V	NB	CB	PC	PT
P1	11.45	19.78	5.87	0.36	9.3	5.01	10.4	492.12
P2	13.10	23.28	6.08	0.55	7.1	4.15	9.3	268.80
P3	10.30	21.85	5.93	0.42	9.4	4.50	10.3	424.68
P4	13.50	25.30	6.24	0.71	9.9	4.88	9.5	457.79
P5	11.30	16.50	6.12	0.37	8.8	4.87	13.0	541.78
P6	10.40	18.15	5.84	0.41	7.7	4.50	10.4	360.56
P7	12.30	22.70	5.65	0.52	11.0	4.09	9.4	414.35
P8	6.73	16.05	5.03	0.15	6.9	5.01	11.6	403.15
P9	13.50	18.86	5.60	0.43	7.9	4.63	10.2	369.00
P10	13.40	20.08	6.63	0.48	10.0	4.84	12.5	609.64
P11	9.10	17.45	5.55	0.25	6.4	4.55	8.9	260.75
P12	11.80	22.60	6.21	0.69	9.8	4.87	9.2	447.63
Mean	11.41	20.22	5.90	0.45	8.7	4.66	10.4	420.86
CV	17.93	14.53	6.98	36.38	16.73	6.64	12.77	24.07
SEm	0.59	0.85	0.12	0.05	0.42	0.09	0.38	29.24
LSD (P<0.05)	2.01	5.34	0.70	0.43	2.16	0.70	1.2	133.72

HT= tree height (m), DBH= diameter at breast height (cm), CS= Crown spread (m), V= tree volume (m^3), NB= number of branches per tree, CB= number of capitulum per branch, PC= number of pods per capitulum, PT= number of pods per tree, CV= coefficient of variance, SEm= standard error mean, LSD= least significant difference, n=10.

The diameter was measured at breast height i.e. 1.37 m above the ground. Height was measured by using a Ravi multimeter at a distance of 20 m from the CPT. In the sloping ground, height measurements were done by using trigonometric principles. Finally, the crown width was measured in two directions right angle to each other.

4.2.3 Seed and pod collection

Ten phenotypically superior plus trees, having a good history of seed production and without insect or pest attack were chosen in each provenance for collection of seeds. Care was taken for that each candidate plus tree was at least 100m apart to ensure maximum genetic variation and also to prevent inbreeding depression (FAO, 1975). Fifty mature pods were plucked from each tree and bulked together for the respective provenance. Collected pods were then air-dried for 30 days; 10 days under direct sunlight and 20 days under shade. Drying of seeds in intact pods may enhance seed maturation (Adms and Rinne, 1981; Banful et al., 2011). Seeds in each provenance were weighed and grouped as per its variation. As lighter weight seeds have low germination potential (Thangjam and Sahoo, 2016), medium to heavy seeds was considered for seed germination and evaluation of seedling traits, in the present study.

4.2.4 Seed and pod morphometric traits (SPT)

A total of 100 pods from each provenance (10 pods/tree x 10 trees) were measured individually for length (PL), width (PW), weight (PWT), number of seeds per pod (SNP) and seed weight per pod (SWP). In each provenance, extracted seeds were bulked and then 100 seeds were selected randomly to measure the length (SL), width (SW), thickness (ST) and weight (SWT). Length of the pod was measured using flexible plastic meter-tap (between basal end and stylar end); width was measured by using digital calliper and weight by using digital weighing balance.

4.2.5 Germination experiment

100 healthy and undamaged seeds drawn from each provenance were considered for seed germination. Seeds were soaked in a 500 ml beaker containing distilled water for 24 hours. These imbibed seeds were then made into 10 replicates of 10 seeds each and placed them in Petri dishes (120 mm diameter) with Whatman No 1 filter paper. Therefore the above experimental design used 1200 seeds (12x100) in 120 Petri dishes (12x10), which were kept in a germinator at a constant temperature (30±2˚C) and 12/12 hr light and darkness for 30 days. Germination percentage, germination energy and mean germination time was then calculated. A seed was considered germinated when its radicle reached 2mm (ISTA, 1999).

Germination Percent (GP) was calculated as the number of germinated seeds as a percentage of the total number of the tested seeds while Germination Energy (GE) was calculated as the percentage of germinated seed obtained at maximum daily germination speed. Germination Index (GRI) and Mean Germination Time (MGT) were calculated following Scott et al. (1984).

4.2.6 Seedling growth and biomass estimation

At a two-leaf stage, 50 seedlings were transferred to poly bags (200 mm x 170 mm) containing sieved garden soil for measuring initial growth parameters. Any kind of organic or inorganic fertilizer, growth hormone or mycorrhiza was not used. These polybags were kept in a completely randomized block design, inside a controlled nursery chamber by maintaining partial shade (50% light intensity). Neither 100% light intensity nor deep shed favour seed germination and seedling growth (Yan and Cao, 2007). Watering was done every alternate day to maintain an ideal condition of soil moisture. The initial seedling growth parameters such as length of the radical or coleoptiles, length of root, collar diameter (CD), etc. were first assessed for 25 plants by destructive process, on 90^{th} day from the two leave stage. Same growth parameters were assessed for the remaining 25 plants on 180^{th} day to see the change in growth. Finally, seedling vigour was estimated by multiplying the seedling length and their respective germination percentage (Abdul-Baki and Anderson, 1973; ISTA, 1985).

4.2.7 Estimation of best performing provenance

Best performing provenance was determined for overall seed characters, pod characters, germinability and seedling vigour. Scores were given to every provenance for every trait based on their morphometric, germinative and growth values. The final ranking for the overall characters was estimated by ranking the aggregate score got by each provenance.

4.3 Statistical analysis

The germination data were subjected to a generalized linear model (GLM) of SPSS (IBM SPSS Statistics 20) for calculating ANOVA. This GLM was also used to compute the maximum likelihood method to measure provenance and environmental variance (error). However, before calculating ANOVA, all the percentage data sets were arcsine transformed to meet the normality assumptions for the variance analysis (Zar, 1996).

To draw an analogy between the magnitude of variation caused by genotype and environment, genotypic coefficient of variance (PCV) and environmental coefficient of variance (ECV) were estimated. Expected mean square for each seed and pod related characters were calculated individually to represent its genotypic variance (σ_{pro}^2) and environmental variance (σ_e^2). Broad sense heritability (h^2) was also calculated to determine the percent of phenotypic variation contributing to the total variation. The following equations were used to calculate PCV, ECV (Burton and de Vane, 1953) and h^2 (Allard, 1960) as:

$$PCV = \frac{\left(\sigma_{pro}^{2}\right)^{1/2}}{Mean} \times 100$$

$$ECV = \frac{\left(\sigma_{e}^{2}\right)^{1/2}}{Mean} \times 100$$

$$h^2 = \frac{\left(\sigma_{pro}^{2}\right)}{\left(\sigma_{pro}^{2} + \sigma_{e}^{2}\right)}$$

Pearson correlation was performed between local climate, geomorphic factors and morphometric traits. Coefficient of variation (CV%) and Least Significant Difference (LSD) was calculated between provenances to determine the amount of variability and differences due to seed, pod and seedling growth traits.

4.4 Results

4.4.1 Seed and pod morphology

Difference in the values of morphometric characters (pods and seeds) were observed among all the provenances. The average values of pod length (PL), pod width (PW), pod weight (PWT), seed weight per pod (SWP), seed number per pod (SNP), seed length (SL), seed width (SW), seed thickness (ST) and seed weight (SWT) are shown in figure 4.3. Variation on average values for pod characters were found as PL varies from 23.6 cm (P1) to 40.3 cm (P7), PW from 2.56 cm (P8) to 3.69 cm (P12), PWT from 19.01 g (P1) to 33.35 g (P5), SWP from 6.4 g (P1) to 17.31 g (P10), and SNP from 9.7 (P1) to 19.6 (P7). Likewise, for seed characters variation was found as: SL from 14.68 mm (P4) to 19.52 mm (P1), SW from 11.6 mm (P4) to 13.61 mm (P1), ST from 5.38 mm (P4) to 7.2 mm (P3), and SWT from 0.5 g (P12) to 1.01 g (P10). Pearson’s correlation coefficient between SPT’s showed significant relationship between SWT and SWP; PL with PWT, SWP and SNP; PWT with SWP and SNP; and SWP with SNP (Table 4.5).

Table 4.3. Effect of seasonal distribution of rainfall and temperature on seed and pod traits of *P. timoriana*

Season	Rainfall		Temperature	
	Pod characters	Seed characters	Pod characters	Seed characters
Winter	PL(-0.57)*, PW(-0.53)*, SWP(-0.52)*, SNP(-0.70)**	ns	ns	ns
Summer	ns	ns	SWP(0.60)*	SWT(0.57)*
Monsoon	PWT(-0.57)*	ns	ns	ns
Post monsoon	ns	ns	SWP(0.55)*	ST(-0.53)*

**two-tailed significance at P<0.05, *one-tailed significance at P<0.05, number within parenthesis represents Pearson's coefficient of correlation, ns = not significant

Table 4.4. Relationship between latitude, longitude and altitude with SPT's, germination and seedling growth characters.

	Pod character	Seed character	Germination characters	Seedling growth characters
Latitude	PWT(-0.69)*,SWP(-0.52)*	SW(0.53)*	ns	SDL1(-0.58)*
Longitude	ns	ns	ns	ns
Altitude	ns	ns	ns	CD2(-0.52)*

Table 4.5. Pearson's correlation coefficient between SPT's of 12 *P. timoriana* provenances

Sl.	SPT	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	SL	0.50	0.01	0.43	0.04	-0.18	0.15	0.21	-0.11	0.52	0.55	-0.21	-0.10	-0.37	0.11
2	SW	1	0.15	-0.23	0.11	0.30	-0.16	-0.21	-0.08	0.16	0.22	-0.41	0.00	-0.44	0.10
3	ST		1	0.02	-0.08	-0.19	0.05	-0.16	-0.35	-0.29	-0.31	-0.18	0.16	-0.10	-0.07
4	SWT			1	0.31	-0.42	0.53	0.86**	0.27	0.55	0.52	0.58*	0.09	0.51	0.12
5	PL				1	0.35	0.76**	0.70*	0.89**	-0.07	-0.10	0.54	-0.04	0.48	0.08
6	PW					1	-0.04	-0.09	0.40	-0.14	-0.18	-0.23	0.13	-0.23	-0.15
7	PWT						1	0.73**	0.72**	-0.09	-0.16	0.59*	0.15	0.51	0.21
8	SWP							1	0.72**	0.34	0.31	0.76**	0.04	0.69*	0.06
9	SNP								1	-0.10	-0.13	0.64*	-0.05	0.58*	-0.04
10	GP									1	0.99**	0.23	0.34	0.14	0.48
11	GE										1	0.18	0.26	0.09	0.44
12	SDL1											1	0.12	0.97**	0.19
13	CD1												1	0.13	0.78**
14	SDL2													1	0.15
15	CD2														1

*Correlation is significant at P>0.05 (2 tailed)
**Correlation is significant at P>0.01 (2 tailed)

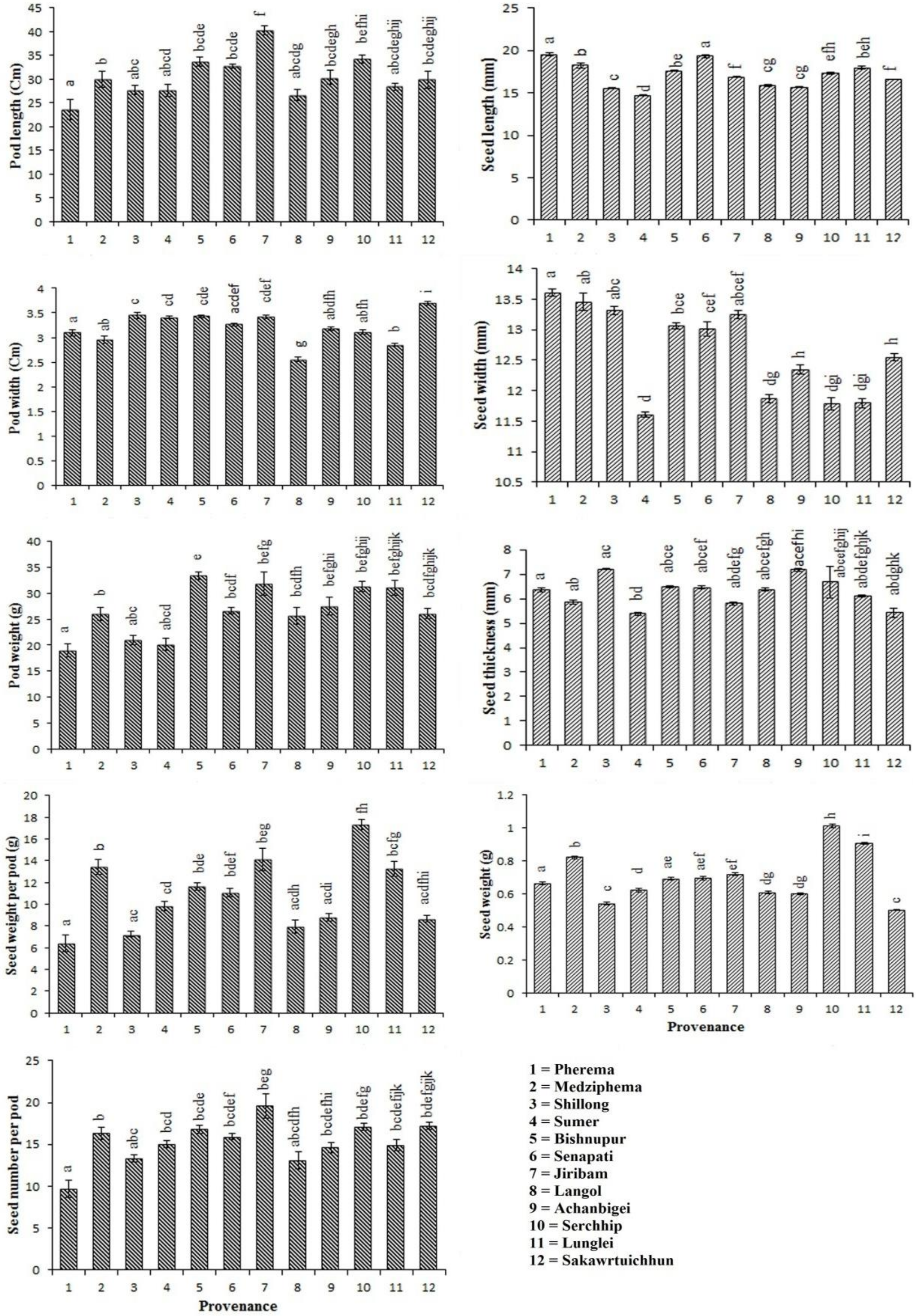

Figure 4.3. Morphometric characters of *P. timoriana* in 12 provenances

SPT's were also found to be affected by the seasonal distribution of rainfall and temperature (Table 4.3). Increase in rainfall during winter and post-monsoon was found to decrease the characters related to pods of *P. timoriana.* On the other hand, high temperature during summer and post-monsoon season increased SWP and SWT. Increase in post-monsoon temperature inversely affects ST. Value of latitude was also found to affect some of the SPT's (Table 4.4). Increase in Latitude increased SW while decreases PWT and SWP.

Table 4.6.Variance component analysis of pod morphometric traits, seed morphometric traits, germination traits and seedling growth traits of *P. timoriana*.

Source of variation	df	Variance components					Expected mean square
Morphometric traits							
Pods		PL	PW	PWT	SWP	SNP	
Provenance	11	17.387	0.095	20.776	10.287	5.794	$\sigma_e^2 + 10\sigma_{Pro}^2$
Error	108	16.199	0.023	17.504	3.254	5.592	σ_e^2
Seeds		SL	SW	ST	SWT		
Provenance	11	2.288	0.535	0.313	0.022		$\sigma_e^2 + 10\sigma_{Pro}^2$
Error	108	0.194	0.065	0.4	0.001		σ_e^2
Germination traits		GP	GE				
Provenance	11	0.067	0.035				$\sigma_e^2 + 5\sigma_{Pro}^2$
Error	108	0.021	0.013				σ_e^2
Growth traits		SDL1	CD1	SDL2	CD2		
Provenance	11	24	0.11	192.676	0.688		$\sigma_e^2 + 25\sigma_{Pro}^2$
Error	288	38	0.032	120.113	0.141		σ_e^2

PL – pod length, PW – pod width, PWT – pod weight, SWP – seed weight per pod, SNP – seed number per pod), SL – seed length, SW – seed width, ST – seed thickness, SWT – seed weight, GP – germination percent, GE – germination energy, SDL1 and CD1 – seedling length and collar diameter at 90 days, SDL2 and CD2 – seedling length and collar diameter at 180 days).

Table 4.7. Broad sense heritability and coefficient of variation for seed germination and seedling growth characters of *P. timoriana*.

Characters	Overall mean	Coefficient of variation(%)		Heritability (%)
		genetic	Environment	
Germination Parameters				
GP%	49.33	1.36	0.43	91.04
GE%	41.08	0.85	0.32	87.87
Seedling growth parameters				
SDL1	60.4	8.11	10.21	38.71
CD1	4.75	6.98	3.77	77.46
SDL2	103.82	13.37	10.56	61.60
CD2	6.69	12.39	5.61	82.99

4.4.2 Seed germination

Effect of provenance on the germination of seeds of *P. timoriana* is shown in figure 4.4. Both germination percentage and germination energy were found to be maximum in provenance 1 (91%, 75%) followed by provenance 7 (83%, 64%). Whereas, the shortest time to germinate (MGT) was found in seeds drawn from provenance 11 (10.19 days) followed by provenance 1 (10.26 days). Values from the variance component analysis (Table 4.6) were used for the calculation of PCV, ECV and ultimately heritability. Role of geographic and environmental factors was found to be small for the germination test (Table, 4.4 and 4.5). Therefore, the total variation in both germination percentage and germination were due to genetic effect (h^2 = 91.04 and 87.87).

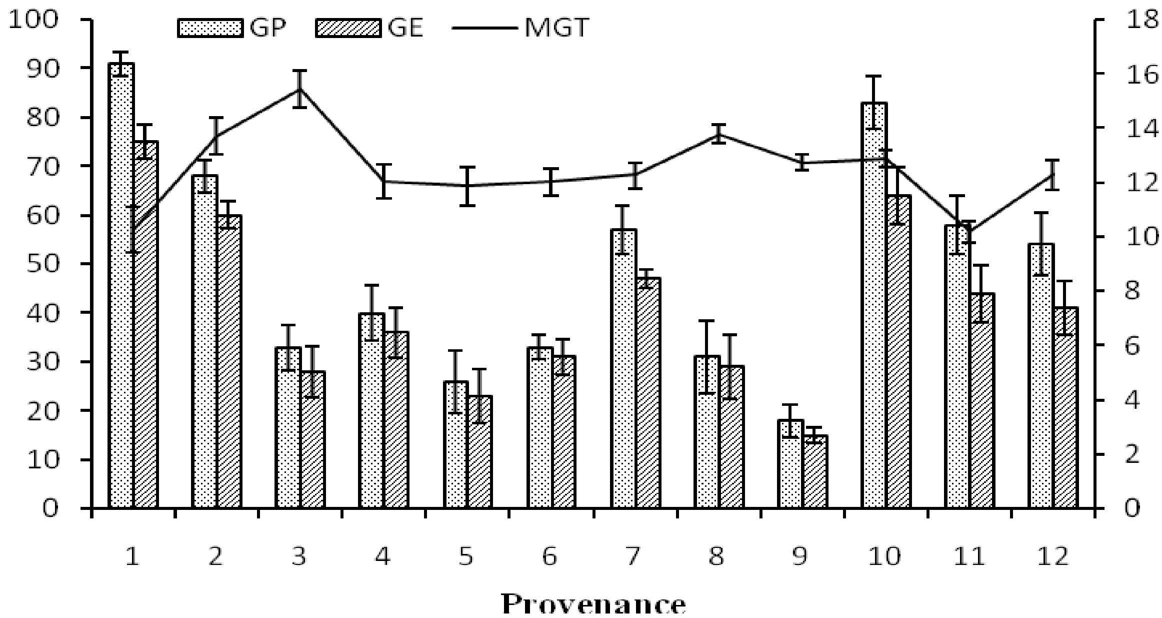

Figure 4.4. Effect of different provenance on germination traits of *P. timoriana*

4.4.3 Seedling growth

Considerable increase in seedling height, collar diameter and seedling vigour were observed from 90th day to 180th day (Table 4.8). Seeds of provenance 10 gave maximum thrust in seedling height and seedling vigour on both 90th and 180th day. However, it was observed that an increase in seedling height does not necessarily follow by its collar diameter. On the 90th day from the two-leaved stage, maximum collar diameter was seen in seedlings from provenance 9 whereas after 180th day it was observed from provenance 1. Among all the sites, five provenances namely P1, P2, P7, P10 and P11 were found to give high vigour seedling.

Correlation analysis between seedling traits with SPT's and germination traits showed significance in SWT, PWT, SWP and SNP (Table 4.5). Geographic factors such as latitude and altitude also affect seedling growth. An inverse relation between latitude with SDL1 and altitude with CD2 were observed in our study (Table 4.4).

Table 4.8. Initial growth parameters of *P. timoriana* as affected by different seed sources in Northeast India

Provenance/ Seed source	Seedling growth parameters						
	SDL1	CD1	SV1	SDL2	CD2	SV2	VI
P1	47.75±0.6	5.27±0.04	4345.07	76.4±0.87	7.89±0.05	395401.19	HV
P2	64.52±1.28	4.69±0.02	4387.63	111.79±2.22	6.65±0.03	298358.98	HV
P3	56.74±1.27	4.58±0.02	1872.42	102.52±20	5.62±0.03	61789.86	LV
P4	56.98±1.15	4.33±0.03	2279.20	100.37±2.22	5.86±0.04	91168.00	LV
P5	53.3±1.1	5.01±0.04	1385.80	91.37±2.12	6.78±0.06	36030.80	LV
P6	54.68±1.14	3.44±0.03	1804.44	87.43±1.52	5.11±0.05	59546.52	LV
P7	70.02±1.97	4.69±0.1	3991.14	120.75±4.17	7.56±0.15	227494.98	HV
P8	62.6±1.46	4.32±0.06	1940.60	111.12±2.80	6.94±0.10	60158.60	LV
P9	59.16±0.99	5.45±0.04	1064.88	102.11±1.96	7.13±0.13	19167.84	LV
P10	72.04±1.02	5.31±0.09	5979.32	126.6±2.01	7.23±0.05	496283.56	HV
P11	67.06±0.82	4.7±0.04	3889.48	113.2±1.41	6.52±0.05	225589.84	HV
P12	61.04±0.45	5.19±0.04	3296.16	102.16±1.06	7.04±0.05	177992.64	LV
Mean	60.49	4.75	3019.68	103.82	6.69	179081.90	
CV	14.79	12.48	50.08	16.63	13.23	86.2	
SEm	0.52	0.03	436.59	0.99	0.05	44560.12	
LSD(P<0.05)	3.72	0.16	1050.21	6.98	0.25	107188.20	

SDL1= seedling length(cm) on 90th day, CD1= collar diameter(mm) on 90th day, SDL2= seedling length(cm) on 180th day, CD2= collar diameter(mm) on 180th day, SV= seedling vigour, VI= vigour index, HV= high vigour, LV= low vigour.

Comparison between seedling length and collar diameter with respect to total variation due to genetic and environment effect showed that percent heritability was more in collar diameter

(77.46%). Increase in broad sense heritability with an increase in the number of days of planting was also seen in both seedling growth and collar diameter (Table 4.7).

Irrespective of the influence of environmental and genetic factors, if one has to choose among the provenances for the overall traits then ranking becomes important. Table 4.9 gives the overall rank of the provenances with respect to their morphological (seed and pod), germination percentage and seedling vigour. P1 gave the best result for seed traits, P7 for pod traits, P12 for GP and P10 for SV.

Table 4.9. Overall rank of the provenances with respect to their morphological (seed and pod), germination percentage and seedling vigour.

Provenance	Seed traits	Pod traits	GP	SV
P1	1	12	6	2
P2	2	6	4	3
P3	8	10	2	8
P4	12	9	12	7
P5	4	2	10	11
P6	3	5	5	10
P7	7	1	8	4
P8	10	11	7	9
P9	9	7	11	12
P10	5	3	8	1
P11	6	8	3	5
P12	11	4	1	6

4.5 Discussion

Each provenance had a particular set of additive factors such as climatic, topographic, biotic and edaphic which influenced the plants to have a distinctive character. The occurrence of *P. timoriana* over a wide geographic range (Hopkins, 1994) is expected to give large diversity controlled by both genetic and environmental coefficients. In the present study, a substantial variation in the seed and pod morphometric traits (SPT's) were observed between provenance, and almost all the variations were of provenance effect (except ST). Genetic control of seed and pod morphometric traits were also discussed in many other tree species (Jayasankar et al., 1999b; Gera et al., 2000; Mkonda et al., 2003). In addition, SPT's were found to have significant correlation with rainfall, temperature and latitude. During winter season (December to February), with scanty rainfall *P. timoriana* showed an improvement in various SPT's such as PL, PW, SWP and SNP. However, the onset of monsoon (June to September) increased SWT in this species. Similarly, temperature too had a discernable influence on some pod and seed characters. Increase temperature in summer (March to May) increased SWP and SWT, while an increased post-monsoon temperature (October to November) increased SWP but decrease ST. Study on correlation of SPT's with seasonal variation of rainfall and temperature was important as many researchers have argued that precipitation and temperature during seed development plays a critical role in the overall seed characters and later on seedling growth (Wulff, 1995). Our study also found significant effect of latitude in determining seed and pod morphology. As latitude increases i.e. from south to north there was an increase in SW while decrease in PWT and SWP. A similar report was given by Vakshayas et al., (1992) in *Dalbergia sissoo* while work is done by Kumar and Toky (1993) on *Albizia lebbeck* gave contradictory result. There was also a strong correlation between PL, PWT, SWP and SNP. Similar findings were reported by Loha et al. (2006) in provenance variation study of *Cordia Africana*. In tree improvement programme correlation of quantitative traits are of significant importance, as the improvement of one trait may cause concurrent change in other traits.

Degree of germinability varies both within and between populations and within and between individuals. Similar findings have been reported for a number of species elsewhere (Gera et al., 2000). Our study also displayed a significant difference in germination parameters between provenances, and the extent of variation was higher for germination percentage than germination energy. Germination of a species is controlled by its genetic origin and also its phenotype, i.e. the local conditions in which the seeds matured. Out of the total variation, 91% of the germination capacity and 87% of the germination energy were attributed to genetic effect. However, the influence of maternal factors such as the position of the seeds in the pod/tree, age of the mother plant, day length, light quality on germinability as observed by Gutterman (2000) could not be ruled out for this species. Under natural condition, one of the important survival strategies to overcome unfavourable condition is by rapid germination and seedling emergence. Among the 12 provenances, P11 and P1 took minimum time for the seeds to germinate. Therefore, the above seeds maybe use to plant in a natural condition where the competition of food in high. It was also observed that none of the SPT's has shown significant correlation with germination percentage and germination energy, therefore the seed and pod morphometric traits have little importance in predicting germinability of the seeds of *P. timoriana*. On the other hand, since the fruits of this species are large and edible the seed and pod morphology could be used for seed dispersal purpose i.e. endozoochory (Jansen et al., 2002).

Many workers have studied provenance variation in seedling length and diameter on different species and concluded their expression is species-specific. For example, Ibrahim (1996) studied seedling diameter at nursery stage for *Faidherbia albida* after 3 months, Sagth and Nautiyal (2001) for *Dalbergia sissoo* after 6 months, Jayasankar et al. (1999a) for *Tectona* *grandis* after 8 months and Loha et al. (2006) for *Cordia africana* after 4 and 8 months respectively. Highest seedling length and vigour was found in P10 for *P. timoriana* in the present study. This area represents a relatively higher seed size and weight than other population and presumably having a higher amount of food reserve contributing to their germination. Yet, another potential determinant used by many plant breeders is the vigour index and in our present study five populations (41.6%) out of twelve seed source resulted in high vigour seedlings with P10 population provided the best seed source in terms of quality seedlings. Low vigour in 58.4% population may be due to inbreeding depression. *Parkia timoriana* is a self-incompatible tree (Endress, 1994), however the absence of important external pollinators like fruit bats (Bumrungsri et al., 2008) in these regions could have resulted in selfing triggered by short distance pollinators such as fly, ants and bees, thereby reducing genetic diversity, vigour and reproductive ability of the plant among population (Koelling et al., 2011). It has been also recommended that diameter and height could be under rather different genetic control, where height is considered to be less sensitive to environmental conditions than diameter (Costa and Durel, 1996). Our result also supports the above recommendation but with contradictory result, as height was more sensitive to environment than diameter.

CHAPTER 5

Effects of different pre-treatments on seed germination and seedling growth of *Parkia timoriana* (D.C.) Merr.

5.1 Introduction

Parkia timoriana (DC.) Merr (Syn *P. javanica*, family Leguminosae) is one of the promising agroforestry tree species of North East India and other South-East Asian countries like Philippines, Malaysia, Thailand, Japan and Vietnam (Salam et al., 1998). It is commonly found in home gardens and shifting agriculture lands in North East India. It is also found in wild state in forests having an altitudinal range from 0-60 to ≥1300 metres above sea level (Devi and Das, 2012).

Various plant parts of this species such as pods, seeds, flowers, young shoots, are consumed by both tribal and non-tribal population of North-East Indian states either in raw form or in various preparations such as salads and curries (Salam et al., 1998). The plant provides a good economic return (Rocky and Sahoo, 2004), besides, it also serves as an important dietary supplement as-well-as medicinal value for curing various ailments (Devi, 2011).

Normally, many trees exhibit seed dormancy so as to have a better chance of survival during unfavourable conditions (Carvalho and Nakagawa, 2000). Like many other leguminous trees, *P. timoriana* have hard-coated seeds resulting in delay germination, hence the seeds show increase germination time and reduced germination energy. Under natural conditions, the seeds may take a much longer period to germinate thus necessitating the use of pretreatments as in many other legumes (Aref et al., 2011; Doran et al., 1983) to fasten germination.

For obtaining optimum germination and seedling vigour, proper conditioning of seeds is nevertheless essential. The importance of pre-treatments for many tree seeds, especially the legumes has been emphasized by many workers (Doran et al., 1983; Tietema et al., 1992; Sahoo et al., 2007). Several pretreatments such as stratification, acid, nicking etc. have been used to trigger germination while some inorganic substrates such as sand, filter paper, vermiculture etc. have been found supporting seed germination and seedling growth of trees. However, information pertaining to the effect of pretreatments on seed germination and initial growth parameters of *P. timoriana* are limited. Therefore, our study may be useful: (1) to define the type of dormancy pertaining in the seeds of this species, and (2) identify the best pre-germination treatment for faster germination, maximum germination percentage and seedling vigour.

5.2 Materials and methods

5.2.1 Seed collection and preparation

The seeds of *Parkia timoriana* were collected directly from naturally grown standing trees during May to June 2015, from Lunglei district of Mizoram (Latitude 23°11′26″N and Longitude 92°45′06″E), having an altitude of 809 m above msl. Twenty-two trees were selected randomly across the terrain for the purpose, and five pods from each tree were harvested manually by using a bamboo pole. Seeds were extracted from the pods successfully by using secateurs. Any infected seed noticed was discarded. All healthy and intact seeds were air-dried and stored at room temperature (28±2°C). Size and weight variation of the intact seeds were examined; the seeds that weigh greater than the average weight were all bulked and from this, a representative sample was taken for the germination experiments. The soil of the seed collection site is acidic (pH 4.64), and having organic carbon of 1.05%.

5.2.2 Seed treatments and scarification

Prior to seed germination, the seeds were subjected to ten pre-treatments *viz.*, (1) seeds were soaked in tap water inside a 100 ml beaker for 24 hours at room temperature (28±2 °C) and then rinsed with distilled water and air-dried; (2) seeds were soaked with 500ppm GA_3 in a 100 ml beaker and kept for 12 and 24 hours, which then finally rinsed with distilled water and air-dried; (3) pre-chilled the seeds at 5°C for 5 days; (4) scarified manually by cutting 1mm of the seed coat at the opposite side of the helium by secateur; (5) seeds soaked in concentrated sulphuric acid (98%) for 1 and 5 minutes, after which the seeds were thoroughly rinsed in tap water and distilled water, and finally air-dried; (6) seeds soaked in boiling distilled water at 100°C and left for 2, 5 and 10 minutes respectively inside a 500 ml flask. A control set of experiment (without any pre-treatments) was also used to compare the result with the treated seeds and for each treatment, 100 seeds were used, replicated five times.

5.2.3 Germination experiments

Treated seeds were sown in top of paper (TOP) media for germination experiment. Whatman No 1 filter paper was used for TOP media. Five replicates each containing 20 seeds for every treatment was used; totalling 1100 seeds (20 seeds x 5 replicate x 11 treatment). The substrata were kept moisten by adding distilled water whenever needed throughout the duration of the experiments. The experiment was laid out in a completely randomized block design. Petri dishes were kept in a growth chamber at a constant temperature (30±2°C) and 12/12 hr light and darkness. Germination was monitored daily and recorded. Seeds were considered germinated when healthy white radicle was seen emerged through the integument (ISTA, 1976).

As the seed breaks dormancy and seedlings emerged, the initial growth parameters were estimated using ISTA (1999) guidelines. The percent germination (GP), mean germination time (MGT), germination energy (GE), germination index (GI) and seedling vigour (SV) was calculated as follows:

a) Germination percentage (GP): the number of germinated seeds as a percentage of the total number of the tested seeds.

GP = (germinated seeds/total tested seeds) x 100 %

b) Mean germination time: Scott et al. (1984) as;

(MGT days): = $\Sigma T_i N_i / S$

Where T_i is the number of days from the beginning of the experiment, N_i the number of seeds germinated per day and S is the total number of seeds germinated.

c) Germination index (GI):

GI = $(G_1/1) + (G_2/2) + \ldots\ldots\ldots + (G_x / x)$

Where G is the germination day 1, 2…, and x represents the corresponding day of germination (Esechie, 1994).

d) Germination energy (GE): the percentage of seed germination obtained at maximum daily germination speed.

e) Seedling Vigor (SV):

SV = Sh x GP

Where Sh is the seedling height and GP is the Germination Percentage.

The initial seedling growth parameters such as length of the radical or coleoptiles, length of root, fresh and dry mass of seedlings, the number of leaves, collar diameter were assessed after 45 days from the two leave stage. The dry weight of the seedling was estimated by oven drying at 60˚C for 48 hours.

5.3 Statistical analysis

Analysis of Variance (ANOVA, 2-way) was carried out using Microsoft Excel to test the effect of different treatments on seed germination and Least Significant Difference (LSD) was used for mean separation. Coefficient of correlation among different plant parameters like root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, number of leaves, collar diameter, root-shoot ratio, biomass and seedling vigour, were also assessed.

5.4 Results

5.4.1 Seed Germination

Germination of seeds in *P. timoriana* was greatly influenced by various pretreatments (Table 5.1). Among these pretreatments, tap water gave maximum seed germination (72%) followed by seed exposure to GA_3 for 24 hours (64%) and stratification (52%). The seeds treated with tap water and GA_3 for 24 hrs, significantly ($P<0.05$) showed an increase in germination over control. The duration of exposure of the seeds to either boiling water or concentrated H_2SO_4 also influenced the germination percent significantly ($P<0.05$). An inverse relation between seedling germination and the duration of treatment was followed in all the treatments (Table 5.1). Nicking though tended to enhance seed germination but it was not significantly different from control.

Table 5.1. Effect of different pretreatments on germination traits of *P. timoriana* under TOP medium

Treatments	TOP medium			
	GP	MGT	GE	GI
tap water	72.00	6.50±0.24	72.00	9.50±0.15
GA_3 12 hour	48.00	5.10±0.22	28.00	13.23±0.11
GA_3 24 hour	64.00	9.30±0.25	44.00	11.55±0.17
Stratification	52.00	9.10±0.33	52.00	6.49±0.07
H_2SO_4 1 minute	20.00	5.60±0.25	20.00	1.81±0.04
H_2SO_4 5 minute	16.00	8.80±0.37	16.00	1.06±0.02
boiling water 2minute	40.00	18.30±0.43	32.00	7.16±0.04
boiling water 5 minute	36.00	11.10±0.42	32.00	4.42±0.05
boiling water 10 minute	20.00	15.80±0.57	16.00	4.25±0.02
nicking	24.00	6.70±0.28	24.00	2.08±0.04
control	20.00	8.00±0.27	20.00	1.75±0.04
LSD ($P<0.05$)		1.69		1.33

MGT = Mean germination time, GI = Germination index, GP = Germination percentage, GE = Germination energy, values are pooled means ±SEM, n=5

5.4.2 Mean germination time

Mean germination time (MGT) was least (5.1 days) in GA_3 for 12 hours, followed by H_2SO_4 for 1 minute (5.6 days) and then by tap water (6.5days). Boiling water treatments took the longest time (18.3, 11.1 and 15.8 days) at 2, 5 and 10 minute exposure respectively and significantly increased MGT compared to the control. On the other hand, H_2SO_4 for 5 minutes (8.8 days), stratification (9.1 days) and GA_3 24 hour (9.3days) treatments had shown little influence on the seed MGT.

5.4.3 Germination energy

The percentage of germination obtained at daily germination speed was found maximum (72%) in case of tap water, followed by cold treatment or stratification (52%) and GA_3 24 hrs (44%). Equal value of GE was recorded in the corresponding treatments: boiling water for 2 minute and 5 minutes (32%), H_2SO_4 1 minute and control (20%), and H_2SO_4 5 minute and boiling water 10 minutes (16%) respectively.

5.4.4 Germination index

Germination index (GI) of the seeds was found in the order: treatment with GA_3 for 12 hours> GA_3 for 24 hours> tap water > boiling water for 2 minute and least when treated with concentrated H_2SO_4 for 5 minutes.

5.4.5 Initial growth parameters

The effect of different pretreatments on initial growth parameters of *P. timoriana* seeds under TOP medium is shown in Table 5.2. Seeds treated with GA_3 for 12 hrs gave the highest result for the following parameters: root length, shoot dry weight, number of leaves, collar diameter, total biomass and seedling length. Similarly, GA_3 treatment for 24 hrs was most favourable in increasing the seedling vigour, while, boiling water found most favourable for increasing seedling length and root dry weight. Control gave the highest Root-Shoot ratio under this media.

Pearson coefficient of correlation showed a significant relationship between all the growth parameters except for collar diameter and root-shoot ratio (Table 5.3).

5.5 Discussion

Seed germination may be influenced by several external and internal factors. Adaption to the prevailing environment causes different species to evolve differently against dormancy. This allows seeds to germinate only when conditions are likely to favour the establishment of a new plant (Bewley, 1997; Hilhorst, 1995; Vleeshouwers et al., 1995; Li et al., 1997; Baskin and Baskin, 2004). The cause and nature of the seed coat impermeability, however, are not fully understood in some plants, but it has been found that under natural conditions and after most pre-treatments the first site at which water penetrates is the stophioles (Harper, 1977). This could be seen as a small raised area close to hilum and is the weakest and the reinforced area of the seed coat. However, variation in the timing and germination percentage of the seeds seen in many species could be attributed to several factors such as the relative position of the seed on the parent plant, micro-environment, quantity of reserve food content and provenance (Owoh et al., 1982; Gutterman, 1982; Gray and Thomas, 1982).

Our study found that simple tap water yielded the highest seed germination when soaked for 24 hours duration. Seeds composed mainly of hydrophilic polymers, with a little amount of osmotically active compound (Obroucheva, 2012) and therefore when the seeds are soaked in water, the water firstly binds to hydrophilic compounds in the cell walls and cytoplasm. And when hydration level reaches 22% (approx), the respiration rate increases, glycolysis and Kerbs cycle are activated, and metabolism of amino acid starts. Further increase in water content (50%) activates protein and mRNA syntheses as well as hydrolysis of stored proteins and starch begins. Hence, when hydration reaches 50-60% all the necessary physiochemical and biochemical activities leads to seed germination. The same was true in *P. timoriana*.

It was further found that GA_3 and stratification helped in seed germination; germination energy and germination index of *P. timoriana*. Reduction in duration for seedling emergence and seedling growth of this species was also reflected by these two treatments. The literature reveals that GA_3 occurs at relatively high concentration in developing seeds but usually drop to a lower level in mature dormant seeds (Yamauchi et al., 2004). It plays a vital role in seed germination in two different stages; first in the initial enzyme induction and second is in the activation of reserve food mobilizing systems. Hence, seed coat treatment of *P. timoriana* seeds by 500 ppm GA_3 might have favoured these two stages, resulting in better germination. Khan (1980) and Yamauchi et al. (2004), also reported the reduction of ABA, following an increase in synthesis of gibberellin and cytokinin is seeds of some tree species after stratification. Thus, GA_3 and stratification are positively correlated which conforms to our findings. Boiling water treatment also gave good result in the present study up to some extent. Increase in temperature might have dissolved the thin waxy coating in this species that prevents water to imbibe the seeds. However, decreased in germination percentage, germination energy and germination index after prolong treatment, might be due to embryo injury triggered by prolong temperature exposure (Otegbeye and Momodu, 2002; Hossain et al, 2005).

In our present study, the effect of H_2SO_4 was minimal in breaking seed coat as is reflected in poor seedling emergence and other growth parameters (Table 5.1 and 5.2). Longer exposure of the seeds to H_2SO_4 might have damaged the embryo of *P. timoriana*. Similar views have been expressed by Aduradola and Adejomo (2005) for *Erythrophleum suaveolens* seeds.

Table 5.2. Effect of pre treatments on various initial growth parameters of *P. timoriana*

Treatments	RL(cm)	SL(cm)	RDW(g)	SDW(g)	NL	CD(mm)	R/S	TB(g)	TSL(cm)	SV	VI
tap water	3.73±0.04	12.30±0.72	0.071±0.010	0.567±0.010	4±0.33	2.96±0.19	0.30±0.002	0.638±0.021	16.03±0.77	1154.16±55.2	HV
GA_3 12 hour	7.50±0.51	18.20±1.01	0.082±0.007	0.598±0.009	5±0.33	3.41±0.04	0.41±0.009	0.68±0.014	25.37±1.45	1233.6±69.7	HV
GA_3 24 hour	4.39±0.16	17.10±2.65	0.076±0.007	0.451±0.014	4±00	3.02±0.04	0.26±0.007	0.527±0.013	21.49±2.59	1375.36±165.7	HV
stratification	4.12±0.30	10.75±2.32	0.036±0.005	0.497±0.010	4±0.33	3.35±0.00	0.38±0.006	0.533±0.012	4.87±2.3	773.24±119.5	HV
H_2SO_4 1 minute	1.90±0.28	7.75±1.18	0.032±0.004	0.067±0.007	3±00	3.23±0.06	0.25±0.010	0.099±0.009	9.65±1.18	193±23.7	LV
H_2SO_4 5 minute	1.50±0.25	5.30±0.33	0.015±0.003	0.055±0.005	3±0.33	3.17±0.03	0.28±0.006	0.07±0.002	6.8±0.58	108.8±9.4	LV
boiling water 2 minute	2.17±0.44	10.96±0.58	0.053±0.005	0.444±0.012	3±0.33	3.20±0.10	0.20±0.006	0.497±0.015	13.13±0.94	367.64±37.6	LV
boiling water 5 minute	3.25±0.38	11.40±1.59	0.088±0.005	0.463±0.012	3±00	3.27±0.06	0.29±0.006	0.551±0.011	14.65±1.95	293±70.2	LV
boiling water 10 minute	4.60±0.87	20.70±2.96	0.090±0.003	0.573±0.008	5±00	3.29±0.04	0.22±0.003	0.664±0.01	25.3±3.83	910.8±76.6	HV
nicking	2.60±0.10	15.57±0.81	0.041±0.004	0.446±0.006	4±00	3.32±0.06	0.17±0.007	0.487±0.004	18.17±0.73	436.08±17.4	LV
control	5.80±1.25	13.80±2.05	0.042±0.005	0.475±0.008	4±0.33	2.95±0.05	0.42±0.005	0.517±0.005	19.6±3.29	392±65.4	LV
LSD ($p<0.05$)	1.83	5.55	0.564	0.564	0.976	0.62	0.564	0.565	6.726	252.9	

RL= root length(Cm), SL= shoot length(Cm), RDW= root dry weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass(g), TSL= seedling length(Cm), SV= seedling vigor, VI= vigor index, HV= high vigor, LV= low vigor, values are pooled means ±SEM, n=5

Table 5.3: Coefficient of correlation between various initial growth parameters of *P. timoriana*

	RL	SL	RFW	RDW	SFW	SDW	NL	CD	R/S	TB
SL	.722**	-								
RFW	.911**	.858**	-							
RDW	.583*	.512	.604*	-						
SFW	.729**	.947**	.859**	.684*	-					
SDW	.319NS	.480NS	.359NS	.880**	.659*	-				
NL	.550*	.529*	.526*	.512NS	.561*	.393NS	-			
CD	-.630*	-.232NS	-.522*	-.101NS	-.135NS	.228NS	.035NS	-		
R/S	.588*	-.129NS	.301NS	.235NS	-.049NS	-.109NS	.210NS	-.095NS	-	
TB	.358NS	.491NS	.396NS	.908**	.672*	.998**	.415NS	.054NS	.220NS	-
SV	.789**	.823**	.785**	.611*	.856**	.527*	.654*	-.099NS	.233NS	.546*

RL= root length(Cm), SL= shoot length(Cm), RFW= root fresh weight(g), RDW= root dry weight(g), SFW= shoot fresh weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass(g), TSL= seedling length(Cm), SV= seedling vigor, VI= vigor index, ** significant at the 0.01 level, * significant at the 0.05 level, NS not significant

CHAPTER 6

Effect of seed mass on germination and seedling vigour of the seeds of *Parkia timoriana* (DC.) Merr.

6.1 Introduction

Every seed comes with a promise to give life on earth. The survival of these seeds, however, is greatly influenced by both biotic and abiotic factors (Grubb, 1977; Harper et al., 1970). Seed mass of species represent a complex adaptive compromise (Harper 1977) and plays a vital role in the establishment of the juvenile phase of a plant's growth curve. Different species give different results (Kapatsa et al., 2014; Maru and Bo, 2015; Owoh et al., 2011) on the effect of seed size on germination. Maximum work on this topic resulted in the more positive effect of larger seed mass, during at least one stage of the life cycle, particularly under stress condition (Harper, 1977; Silvertown, 1989; Manga and Yadav, 1995), which might be because of the larger food reserve present. Larger food reserve in seeds may permit more pre and post photosynthetic growth of seedlings and this in turn, may contribute to better growth and survival in later stages (Harper and Obeid, 1967; Stanton, 1984). However, negative relationship between seed mass and relative growth rate reported across species (Reich et al., 2003; Wright and Westoby 1999; Castro et al., 2008) cannot be ruled out. This negative relationship helps the small or lightweight seeds with less food reserve to compensate and survive unlike the initial advantage conferred to heavy seeded species (Norgren, 1996; Paz et al., 1999). Therefore, this association may be considered as a determinant of plant demography and community composition.

Habitat or microsite variation also influence the mass, size and health of the seedling population in addition to other intrinsic characters of the seeds. The understorey of the forest, where light is limiting is occupied by competitive and tolerant seedlings produced from heavy and large seeds, while light and small seeds with faster germination inhabit the openings. An alternate temperature of 17.5˚/ 30˚C was found to be more favourable (Fabio et al., 2010; Birendra et al., 2013) for the seeds of *Parkia roxburghii* (Sahoo et al., 2007) to get germinated. This condition closely resembles an open forest and hence seeds of all size may germinate, while to a closed canopy forest type, seeds with larger food reserve of this species might be favourable.

Parkia timoriana is one of the important leguminous tree species reported to be found only in the north-eastern states of India and some other parts of south-east Asian countries (Hopkins, 1983). The pod, flower and seed of this tree is a delicacy and consumed either raw or cooked. Hence, these plant products provide a stable flow of cash income to the farmers of the region (Rocky and Sahoo, 2002). Reports of its use in traditional medicine are also well known (Khan and Yadava, 2010).

Effect of seed mass on germination and early growth has rarely been analyzed. However, no related work as such has been reported for *Parkia timoriana* tree seeds. The objective of the present study was to see if there is any difference in seed weight derived from different provenance and if difference is there, whether seedlings derived from heavier seeds have more regeneration potential. Seed scientist and farmers could utilize this information to create a seed orchard or a plantation farm in less time and with more success.

6.2 Materials and methods

6.2.1 Study species

Parkia timoriana (D.C.) Merr is a leguminous tree species in late-successional stage. Flowers, pods and seeds of this plant are edible; therefore it is mostly grown in homestead gardens. It attains a height up to 20 m in plain and 12 m in hills, distributed across the Northeastern states of India with an altitudinal range up to 1300 m asl. Under natural condition, they are mostly pollinated by bats, bees and ants, and after the seeds become mature they prefer moist shady place to germinate.

6.2.2 Seed source

Mature pods were collected from twenty randomly selected trees of *P timoriana* at Sakawrtuichhun provenance of Mizoram, India (23˚45′33″ N, 92˚40′25″ E, 829m asl). These pods were then let dry by keeping under direct sunlight for 10 days and then under shade for 20 days. Extraction of the seeds was done manually by using secateurs and seeds with insect and fungal attack were discarded at the same time. These extracted seeds were then bulked together and were resorted into three categories based on their seed weight as light (<0.5g), intermediate (0.5 to 0.7 g) and heavy (> 0.7 g) respectively, for determining seed germination and seedling growth. Seeds of each class were soaked separately in distilled water at room temperature (28±2˚C) for 24 hours. Soaked seeds were sown separately in 20x17 cm polythene bags containing sieved (1mm) garden soil and watered every alternate day. Daily records were kept until seedlings ceased to emerge (30 days). A seed with a healthy white radical of about 2mm protruding through the integument was considered germinated. The seedlings were allowed to grow for three months for growth-related studies.

The following germination parameters were determined:

- Germination percentage (GP); the number of germinated seeds as a percentage of the total number of the tested seeds is given as;

  GP = (germinated seeds/total tested seeds) x 100 %

- Mean germination time is given according to Scott et al. (1984) as;

(MGT days) = $\Sigma T_i N_i$ / S

Where T_i is the number of days from the beginning of the experiment, N_i the number of seeds germinated per day and S is the total number of seeds germinated.

- Germination Index (GRI): it was calculated for each treatment using the following equation:

  GRI = $(G_1/1) + (G_2/2) + \ldots\ldots\ldots + (G_x / x)$

  Where G is the germination day 1, 2…, and x represents the corresponding day of germination (Esechie, 1994).

- Germination energy (GE): it was calculated as the percentage of seed germination obtained at maximum daily germination speed.

- Seedling vigour (SV): it was calculated by multiplying the seedling length and germination percentage.

6.2.3 Biomass related growth measurement

Measurements are done every 15^{th} day starting from the two-leaved stage of the germinated seedlings. Three seedlings each were randomly selected, uprooted without damage and then taken fresh weight. Further, they were kept inside an oven and dried at 80˚C for 24 hours. The dried samples were taken and weigh again in an electronic balance. These data were used to calculate the following parameters; Relative Growth Rate (RGR), average growth rate AGR) and Root/Shoot (R/S) ratio.

Offiong (2008) defines the relation as:

$$RGR(g/15days) = \frac{LnTDM2 - LnTDM1}{t2 - t1}$$

$$AGR(g/15days) = \frac{TDM2 - TDM1}{t2 - t1}$$

Where; TDM1 = Initial total dry weight; TDM2 = Final total dry weight

t1 = Initial time; t2 = Final time; Ln = Natural logarithm

6.3 Data analysis

Individual seed weights were determined by weighing 255 seeds (45 damaged seeds were discarded) and then a frequency distribution was derived. From this distribution, normality was compared and tested by the K-S test. Relationship among seedling length, collar diameter and biomass for each class of seed mass namely, light, intermediate and heavy were also analysed by using ANOVA. Further calculation for linear regression followed by a regression equation was also computed for the above relationship.

6.4 Result

6.4.1 Seed weight distribution

The distribution pattern of seed weights as calculated from the frequency distribution of 255 seeds did not show lognormal distribution (K-S test: $P<0.05$, $d = 0.163$, $n = 255$). Seed weight (n=255) varied from 0.39g to 0.81g (mean: 0.61g ± 0.01g). Among the weight class, mid-weight (0.5 to 0.69g) seeds made up 56.47% of the total population followed by heavyweight (23.14%) and then by a lightweight (20.39%).

6.4.2 Seed germination

The total germination of *P. timoriana* seeds was 84.62%; 78.85% of lightweight seeds, 41% of intermediate weight seeds and 42% of heavyweight seeds (Table 6.1). Germination started on the 8th day and ended on 19th day for lightweight seeds, on 5th day and 20th day for intermediate weight seeds and on the 8th and 25th day for heavyweight seeds (Figure 6.1). Seed weights were positively correlated with germination time (Figure 6.2).

Table 6.1. Germination behaviour of light, intermediate and heavy seeds of *P. timoriana.*

Seed weight class (g)	Number of seeds shown	Number of seeds germinated	Number of days for initiation of germination	Mean germination time(±SE)	Germination index	Germination energy
Light (<0.5)	52	41 (78.85%)	4	10.62±0.82	30.5	75
Intermediate (0.5-0.7)	52	44 (84.62%)	5	12.87±0.35	24.35	80.77
Heavy (>0.7)	52	47 (90.38)	9	17.11±0.34	21.74	86.54

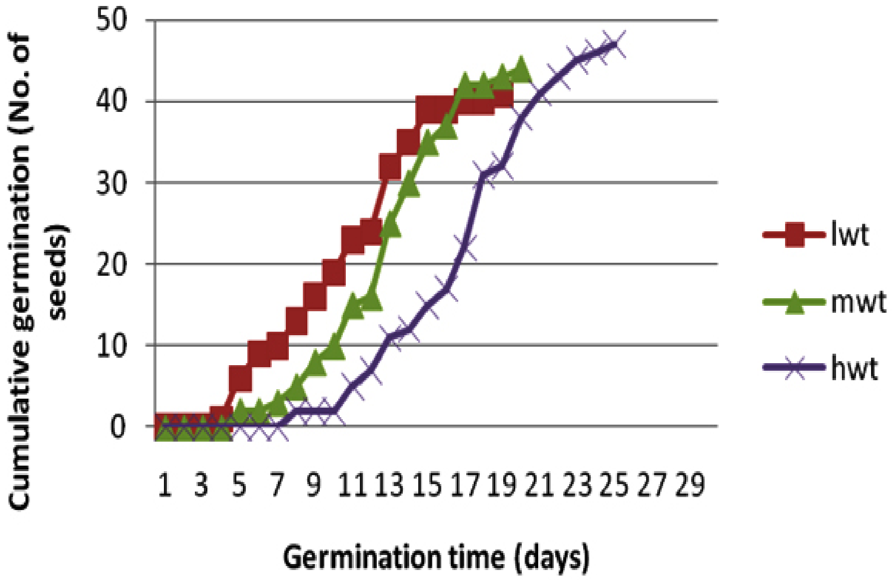

Figure 6.1. Germination curve of light, intermediate and heavyweight seeds of *Parkia timoriana.*

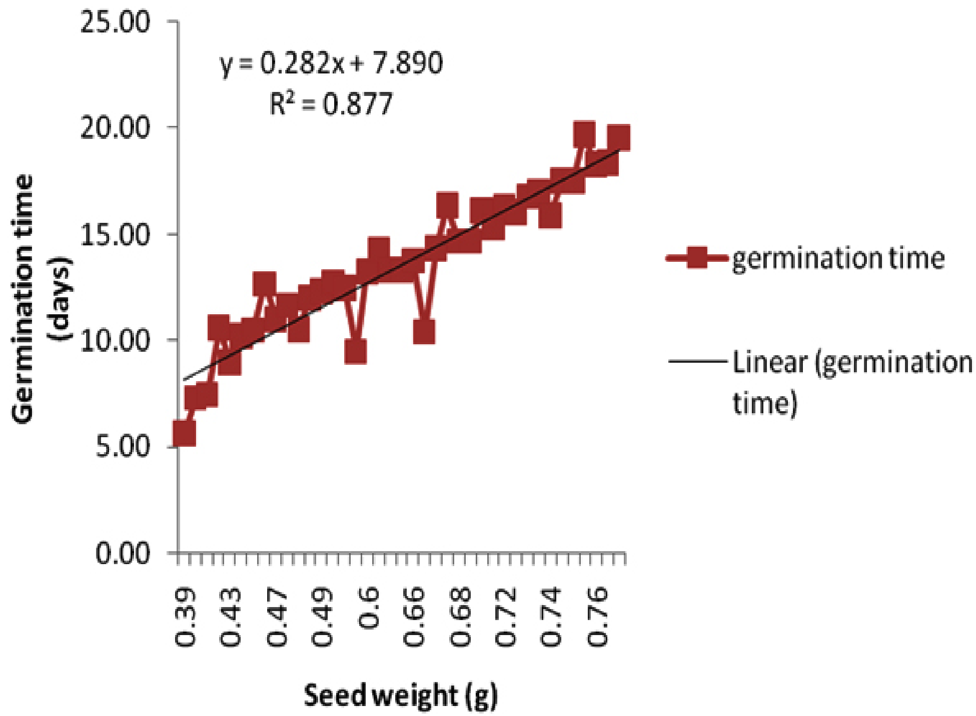

Figure 6.2. Relationship between seed weight and germination time in *P. timoriana*

6.4.3 Seedling growth parameters and their relationship

Significant positive relationship ($P<0.05$) between seedling length, biomass and collar diameter was found for all the three seed weights (Table 6.2). While the comparison of the seed weights regarding the seedling parameters showed no significant difference. A gradual decrease from heavyweight to lightweight was seen in all the parameters apart from the collar diameter (Table 6.3).

Table 6.2. Relationship between seedling length, biomass and collar diameter after 90 days of growth for different weight classes.

Seed weight	Seedling length (sdl)	Biomass (bms)	Collar diameter (cd)	Regression equation	P value
light	49.43±2.43	1.66±0.17	3.90±0.13	sdl=4.29bms+10.33cd	5.31E-07
intermediate	54.56±2.76	1.809±0.14	4.05±0.10	sdl=3.61bms+10.88cd	6.19E-07
heavy	57.90±5.07	1.82±0.35	4.00±0.21	sdl=31.62bms+2.87cd	1.57E-05

Table 6.3. Corresponding growth parameters observed after 90 days for the three weight class; light, intermediate and heavy.

seed weight (g)	root length (cm)	shoot length (cm)	root dry weight (g)	shoot dry weight (g)	no of leaves	collar diameter (mm)	seedling vigor
Light(<0.5)	19±1.53	30.43±.92	0.27±.03	1.39±.14	5.33±.33	3.90±.13	3897.556
Intermediate (0.5- 0.7)	21.33±1.67	33.23±1.40	0.27±.02	1.54±.12	5.67±.33	4.05±.10	4617.149
Heavy(>0.7)	21.50±1.32	36.40±3.91	0.28±.04	1.54±.32	6.00±0	4.00±.21	5233.002

Relative growth rate (RGR), average growth rate (AGR) and root shoot (S/R) ratio as determined by destructive method, revealed a general trend. RGR and AGR increase with an increase in weight, while the reverse is true for R/S (table 6.4). Seedling vigour also has shown positive relation with increasing seed weight and the number of days (figure 6.3).

Table 6.4. Effect of seed size on relative growth rate (RGR), average growth rate (AGR) and root to shoot (R/S) ratio of *Parkia timoriana*.

Treatments	RGR (g/15days)	AGR (g/15 days)	R/S
Light	0.136	0.2	2:10.5
Intermediate	0.174	0.28	2:9.8
Heavy	0.179	0.33	2:9.5

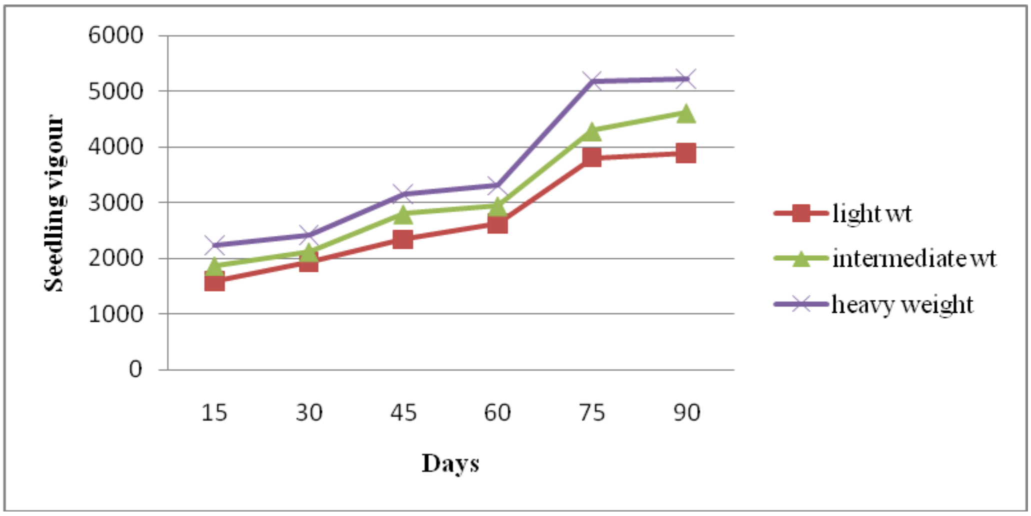

Figure 6.3. Relationship between seedling vigour, seed weight and time in days, taken alternately after every 15 days interval.

6.5 Discussion

Lighter seeds with lesser food reserve can germinate and grow if the competition for light, food and space is favourable. On the other hand, large food reserve is necessary to survive under harsh condition (Howe and Richter, 1982). Seed mass may also be influenced by the position of seed in the pod. In *Parkia timoriana*, because of the difference in nutrient allocation duration during pod filling, seeds from the middle portion of the pod gave heavier weight comparing the top and the bottom region. Longer the duration of pod filling. Bigger and heavier will be the seeds and vice versa (White and Izquierdo, 1989).

Germination and early growth of *P. timoriana* seedlings were significantly affected by the weight of maternal seeds. The better germination exhibited by the heavier seeds could be the result of greater availability of food reserves in heavy seeds (Offiong, 2008; Tripathi and Khan, 1990; Ke and Werger, 1999; Khan et al., 1999; Khan and Uma Shankar, 2001). However, lightweight seeds took less time to germinate than the heavy one. This could be due to thinner seed coat, which is in agreement to the report given by several authors on other tropical tree species (Khan et al., 1999; Marshall, 1986; Murali, 1997).

Seed mass and seed size can be used as an important tool for predicting germination and seedling growth (Oni and Bada, 1992; Simmone et al., 2000). Similarly, a close association among leaf, pod and seed size in beans (Duarte, 1972) are also well documented. These results are in agreement with our present study which showed strong correlation among the seed weights and the seedling parameters. The assumption of common genetic influences on homogenous tissues could be a possible explanation for the above relationship. This means that homology between, leaf, stem, seed etc., may be due to the fact that at the early primordial stage every organ is initiated in a similar manner from an apical meristem.

P. timoriana seedlings from heavy seeds gave better height, leaf number and dry matter yield than seedlings from intermediate and lightweight seeds. In addition to this, the positive correlation between seed weight and seedling vigour put forward the more competitive nature of heavyweight seeds (Bonfil, 1998; Long and Jones, 1996).

Larger food reserve in the heavy seeds could be given credit for the initial superior growth that was observed in *P. timoriana*. This might have accounted for the early comparative growth advantage in the seedlings. A positive correlation between seedling length, biomass and seed mass (Simmone et al., 2000) as reported by seed researchers highlight the above experimental result. Report of superior growth in *Anacardium occidentale* from larger nuts than the smaller one (Faluvi, 1986) also supports this relation.

CHAPTER 7

Seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* (DC.) Merr.

7.1 Introduction

Parkia timoriana (DC) Merr. (Family: Mimosaceae) is one of the well known multipurpose tree species of tropics and subtropics and has a wide distribution in North East India and other South-East Asian countries like Indonesia, Japan, Malaysia, Philippines, Thailand, Vietnam and Northeast India (Salam et al., 1998). This is the only species of Parkia, which is found in both sides of Wallace's line, primarily distributed in the evergreen rain forest, moist mixed deciduous and dry evergreen forests. Altitudinal variation of this species usually range between 0 to 600 m, most common up to 300 m but rarely reaches 1300 m in North East India and Bangladesh, and up to the upper limit of dipterocarp forest in Borneo (Hopkins, 1994).

In India the species is grown mostly in home gardens and fallow lands of shifting cultivation, and the species is of high demand for dietary supplement and the flower and fruits of this species have immense nutritional and medicinal properties (Samuel et al., 2010; Ong et al., 2011; Rathi et al., 2012). However, the tree is vulnerable to the pest insect *Cadra cautella*, a moth whose larva bores into the seed to pupate, feeding on the seed interior and filling it with webbing (Thangjam et al., 2003). *Parkia biglobosa,* another species of this genus in Africa have shown lack of regeneration and ageing of the stands, which might result in complete disappearance over time (Nikiema, 1993; Quedraogo, 1995; Bouda and Nikiema, 1996; Ræbild et al., 2012). Overexploitation, insect infestation, shortening of the fallow period and drier climate could cause complete disappearance of this species unless immediate measures are undertaken (Nikiema, 1993; Teklehaimanot, 2004; Roy, 2016). Above anthropogenic and insect infestation could somehow be controlled but the climatic effect is very difficult to regulate. Thus, the present study aims to screen and select the suitable seed source of *P. timoriana* and to find up to what extent the variability between sources reflects climatic factors. Many workers have tried to find the relationship between seed source or seed zones on germination and growth both within species and between species on many species (Xu et al., 2015; Palinikumara et al., 2015; Aigbe et al., 2016; Moya et al., 2017), however, very little quantitative estimates regarding the role of species' adaptation on different climates is done on this genus and none on this species. The paper is designed using climatic model of Thornthwaite (1948) climatic classification and further mapping with ArcGIS interpolation tool, which fit the provenance related climatic data of *P. timoriana* under various agroclimatic zones.

Many workers have reported that information on morphological and genetic variation of seed and pod characters are very important to provide the better source of seed for afforestation and tree improvement programme (Tomar and Rattan, 2012; Fredrick et al., 2015; Gupta et al., 2016). This paper tries to prove the hypothesis that there is significant variation in quantitative traits of *P. timoriana* along different agro-climatic zones.

7.2 Materials and methods

7.2.1 Site selection and zonation

Parkia timoriana (DC) Merr. was identified using the identification key of the species given by Hopkins (1986, 1994). The *P. timoriana* growing population in Northeast India were first extensively surveyed from the market during December to March (pod harvesting season) followed by their occurrence and distribution in natural stands and home gardens. Based on abundant availability and distribution, 12 seed source covering four northeastern states (viz. Manipur, Meghalaya, Mizoram and Nagaland) were selected for the study. These seed sources were further clustered into four agroclimatic zones (viz. perhumid, humid, subhumid and arid) following Thornthwaite (1948) climatic classification (Figure 7.1). Various agroclimatic variables such as potential evapotranspiration (PET), precipitation, mean monthly temperature of each seed source was considered for calculating moisture index (MI) and annual heat index (I) following Thornthwaite (1948) as follows:

$$\text{Annual heat index, I} = \sum_{i=1}^{12} \text{i} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, $\text{i} = \text{monthly heat index} = \left(\frac{t}{5}\right)^{1.514}$ and t = mean monthly temperature (˚C)

$$\text{PET} = 1.6\left(\frac{10.\text{t}}{\text{I}}\right)^{\alpha}.\frac{\text{L}}{12}.\frac{\text{N}}{30} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, $\alpha = 675\,.10^{-9}.\text{I}^3 - 771\,.10^{-7}.\text{I}^2 + 1792\,.10^{-5}\,.\text{I} + 0.49239$

L = the theoretical sunshine hours for each month

N = number of days for each month.

$$\text{MI} = \frac{100\text{s} - 60\text{d}}{n} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, s = surplus water, which is defined as the sum of the monthly difference between precipitation (P) and PET for those months when P exceeds PET (cm)

d = water deficiency, which is defined as the sum of the monthly difference between PET and precipitation (P) and for those months when PET exceeds P (cm)

n= water need, which is the sum of monthly values of PET for the surplus of deficiency months (cm)

The MI derived thus was interpolated to generate a respective agro-climatic map by using ARCGIS interpolation tool (Childs, 2004). Interpolation is a spatial analysis technique in which values are predicted by averaging the known point values. The moisture index values of sampling sites in each district were used to generate a continuous surface giving us a predicted value of agro climate for the entire state. The climatic data for the calculation of PET and MI were collected from the Indian Meteorological Department sub-stations, Krishi Vigyan Kendra (KVK) stations and state statistical department, of the respective state.

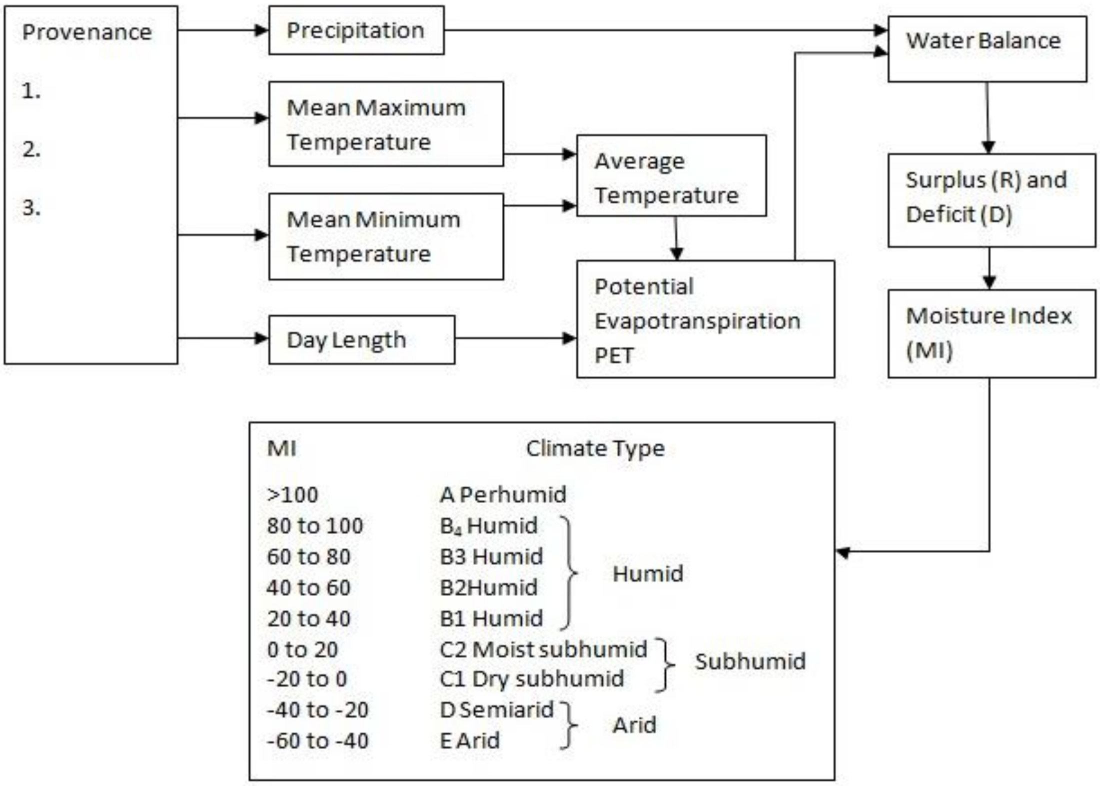

Figure 7.1. Flowchart showing zonation of the provenance of *Parkia timoriana* following Thornthwaite climatic classification

7.2.2 Pod collection and seed extraction

Twenty mature pods each from twelve years old ten candidate trees were collected from twelve provenances representing four states of northeast India i.e. Manipur, Meghalaya, Mizoram and Nagaland. These pods were harvested manually by using a scythe made from a long bamboo pole. Collected pods were then air-dried for 30 days; 10 days under direct sunlight and 20 days under shade. Drying of seeds in intact pods may enhance seed maturation and seed dry weight accumulation consequently increasing viability (Adams and Rinne, 1981). Measurement of the length of pod was done by using a measuring tape, while widths were measured by using a digital calliper. Weights of pods and seeds were taken in a digital weighing balance. Extraction of the seeds was done manually by using secateurs and seeds with insect and fungal attack were discarded at the same time.

7.2.3 Seed Germination and seedling growth traits

After extraction, seeds from each zone were soaked in a 1000ml beaker filled with distilled water for 24 hours. Soaking of seeds in water before sowing enhances germination by increasing osmosis and oxygen intake (Obroucheva and Antipova, 1994; Thangjam and Sahoo, 2017) and also separate viable seeds from non-viable one. These seeds were then sown separately in polythene bags (20X17 cm) containing sieved (1mm) garden soil. Watering was done every alternate day and daily records were maintained until 30 days or till seedlings ceased to emerge. A seed is considered as germinated when a healthy white radical of about 2mm length gets protruded through the integument. The seedlings were allowed to grow till 90 days so as to assess various germination attributes such as germination percentage (GP), mean germination time (MGT), germination index (GI), germination energy (GE) and seedling vigour (SV). GP was calculated as the number of seeds germinated to the total number of seeds sown, expressed as percentage. MGT was calculated following Scott's equation (Scott et al., 1984) as, $MGT = \Sigma T_i N_i / S$ (where, T_i is the number of days from the beginning of the experiment, N_i the number of seeds germinated per day and S is the total number of seeds germinated). GI was calculated following Esechie (1994) as, GI = $(G_1/1)$ + $(G_2/2)$ + ……..+ (G_x / x) (where, G is the germination on day 1, 2…, and x represents the corresponding day of germination). GE was obtained as GI at maximum daily germination speed and SV was calculated by multiplying GP with the seedling length.

Measurements were done on every 15th day interval starting from the two-leaved stage of the germinated seedlings till 90th day. Growth parameters that are assessed include: shoot length, root length, shoot dry weight, root dry weight, collar diameter, total biomass, relative growth rate (RGR), average growth rate (AGR) and seedling vigour. RGR and AGR were calculated following Offiong (2008) as

$$\text{RGR (g/15 days)} = \frac{\text{LnTDM}_2 - \text{LnTDM}_1}{t_2 - t_1}$$

$$\text{AGR (g/15 days)} = \frac{\text{TDM}_2 - \text{TDM}_1}{t_2 - t_1}$$

Where;

TDM_1 = Initial total dry weight, TDM_2 = Final total dry weight

t_1 = Initial time, t_2 = Final time

L_n = Natural logarithm

7.3 Statistical analysis

Both simple and multiple regressions were performed to examine if the regeneration and growth traits follow clinal variation and to see if the variation follows climatic differences. These analyses were also used to derive a polynomial equation depicting the relationships between various traits in an agro climate. Two-way analysis of variance (ANOVA) was performed to understand if there were significant differences among agroclimatic zones for the seed and pod traits. The coefficient of variation (CV) was calculated by dividing the zonal standard deviation of a given trait (σ_{prov}) by the overall average of the zone for that trait ($\overline{X}$). This was determined between the sampled populations to measure the degree of genetic differences.

7.4 Result and discussion

7.4.1 Agroclimatic zonation of seed source

Discernable variations in temperature, rainfall, PET and MI were observed among the twelve seed source of *P. timoriana* (Table 7.1). The moisture index (MI) ranged from -39.42 unit (Jiribam, Manipur) to 237.46 unit (Shillong, Meghalaya). These regions covered all four major agro-climatic zones; arid, subhumid, humid and perhumid. Minimum MI or place with maximum aridity was seen in Jiribam (-39.42), followed by Medziphema (-35.73). These regions despite having average rainfall have high potential evapotranspiration (PET) hence resulted in high aridity. Further, interpolation of the moisture index data for the twelve sample points using ArcGIS showed minor variability in prediction of agroclimatic range from that of the observed Thornthwaite zonation. This might be due to lesser number of raster points taken which decrease the probability for prediction. Therefore, a number of uniformly distributed point samples covering the entire *P. timoriana* population of northeast India is recommended for making a robust agro-climatic model relating agro climate with various qualitative and quantitative characters of the species.

Table 7.1. Climatic variables of 12 seed source of *Parkia timoriana* as per Thornthwaith (1948).

Sl. No.	Seed source	State	Temperature (˚C)	Rainfall (cm)	PET (cm/year)	MI	Code	Agro climatic zone
1	Pherema	Nagaland	24.29	322.37	143	80.33	B4	Humid
2	Medziphema	Nagaland	28	117.97	141.1	-35.73	D	Arid
3	Shillong	Meghalaya	17.43	353.05	82	237.46	A	Perhumid
4	Sumer	Meghalaya	21.54	306.3	106.7	123.68	A	Perhumid
5	Bishnupur	Manipur	20.47	123.69	101.8	-3.45	C1	Subhumid
6	Senapati	Manipur	21.82	153.66	106.5	24.4	B1	Humid
7	Jiribam	Manipur	28.58	194.23	252.8	-39.42	D	Arid
8	Langol	Manipur	21.08	148.21	105.6	1.69	C2	Subhumid
9	Achanbigei	Manipur	23.75	159.12	134.1	15.84	C2	Subhumid
10	Serchhip	Mizoram	25.17	220.76	156.5	11.83	C2	Subhumid
11	Lunglei	Mizoram	25.58	308.04	164	57.96	B2	Humid
12	Sakawrtuichhun	Mizoram	24.92	254.8	151.5	3.4	C2	Subhumid

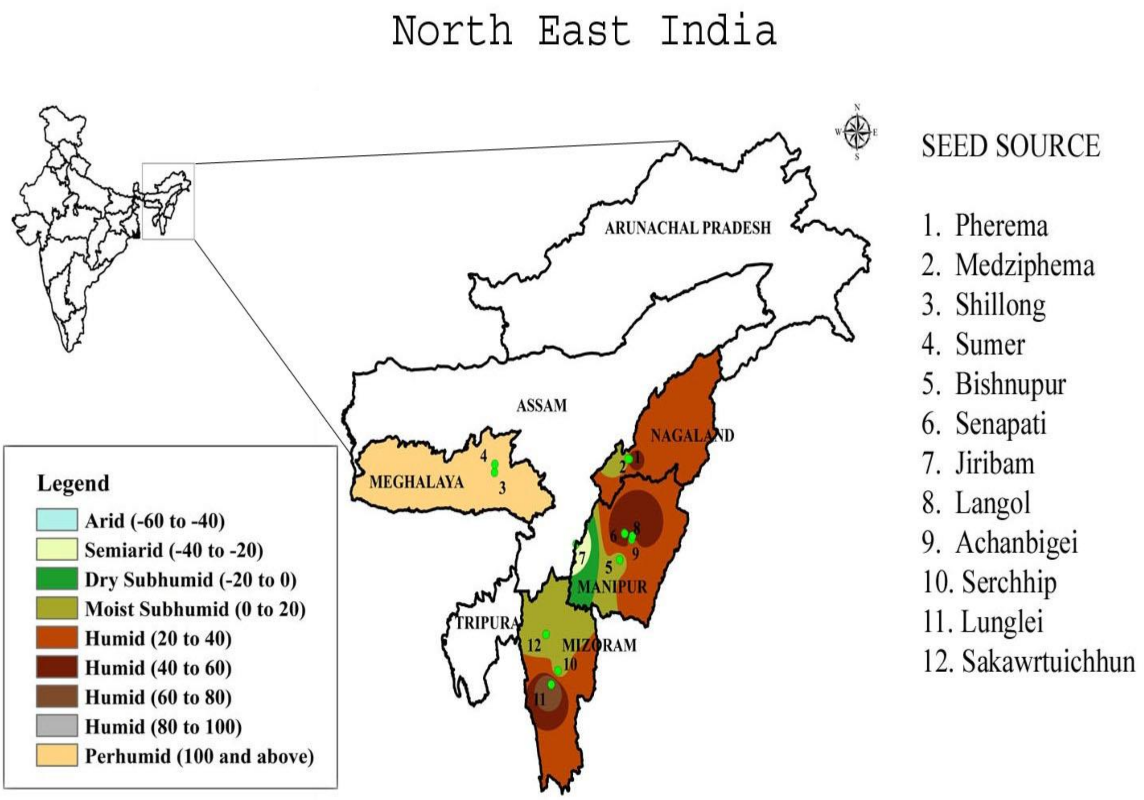

Figure 7.2. Map showing zonal distribution of *Parkia timoriana*, drawn using Arc GIS interpolation tool

7.4.2 Effect of agroclimatic zones on seed and pod characteristics

A high significant difference (P<0.001) was found for the seed and pod traits between the agro-climatic zones of *Parkia timoriana* in the Northeastern states of India (Table 7.2). On the other hand, no significant difference between blocks was found. The highest coefficient of variation between the zones was observed in pod length (CV = 22.3%), closely followed by seed weight per pod (CV = 22.1%) and least in pod width (CV = 6%). Polynomial regression curve drawn for pod and seed characters against agroclimatic zones (Fig. 7.3, a to f) showed a gradual increase in pod length, pod weight, seed weight per pod, seed number per pod and 1000 seed weight, as one moves from perhumid to the arid zone. However, pod width showed higher values in perhumid and arid zones than the humid and subhumid zones. Polynomial regression was used instead of linear as it represents a number of under-fitting data. Figure 7.3 (a & f) represented maximum under-fitted curve when it was drawn with a simple linear regression equation. In addition, higher regression coefficient and lower root mean square error was associated with a polynomial curve than a linear curve.

Table 7.2. Analysis of variance on seed and pod characters of *Parkia timoriana* due to agro-climatic zones

Traits	Source	SS	df	MS	F ratio	P value	CV%
Pod length	Between zones	2507.969	3	835.99	39.88	<0.0001	22.3
	Among zones	1249.412	59	21.18	1.01	0.466808	
	Error	3710.272	177	20.96			
Pod width	Between zones	7.354125	3	2.45	29.65	<0.0001	6
	Among zones	3.557975	59	0.06	0.73	0.919831	
	Error	14.62993	177	0.08			
Pod weight	Between zones	1591.709	3	530.57	19.05	<0.0001	11.1
	Among zones	1246.087	59	21.12	0.76	0.890859	
	Error	4928.679	177	27.85			
Seed weight pod^{-1}	Between zones	1054.153	3	351.38	42.77	<0.0001	22.1
	Among zones	201.5135	59	3.42	0.42	0.999917	
	Error	1454.173	177	8.22			
Seed number pod^{-1}	Between zones	739.1167	3	246.37	31.2	<0.0001	13.2
	Among zones	462.9833	59	7.85	0.99	0.497838	
	Error	1397.883	177	7.90			
1000 seed weight	Between zones	1322665	3	440888.4	38.13	<0.0001	12.3
	Among zones	464891.9	59	7879.524	0.68	0.955842	
	Error	2046396	177	11561.56			

The speculation of this clinal pattern is very important to study as they deal with initiation and cessation of growth that plants have adapted to a spatially different environment. Few works done in India on the clinal response or provenance effect on *Dalbergia sissoo* (Singh and Bhatt, 2008), *Pinus roxburghii* (Ghildiyal et al., 2009) and *Jatropha curcas* (Ginwal et al., 2005; Ghosh and Singh, 2011) pointed out the significant effect of environmental adaptation and genetic effect on defining various qualitative and quantitative traits. Our study also found that the value of most of the studied traits increases as sampling goes from perhumid to arid zones (Fig. 7.3). These zones correspond to places having very high rainfall and potentially lesser evapotranspiration like Shillong (Perhumid) to a place having lesser rainfall with high aridity and temperature like Jiribam (Arid). A similar report was given in another species of Parkia (*P. biglobosa*) in the Sudano-Sahelian zone of West Africa (Quedraogo et al., 2012).

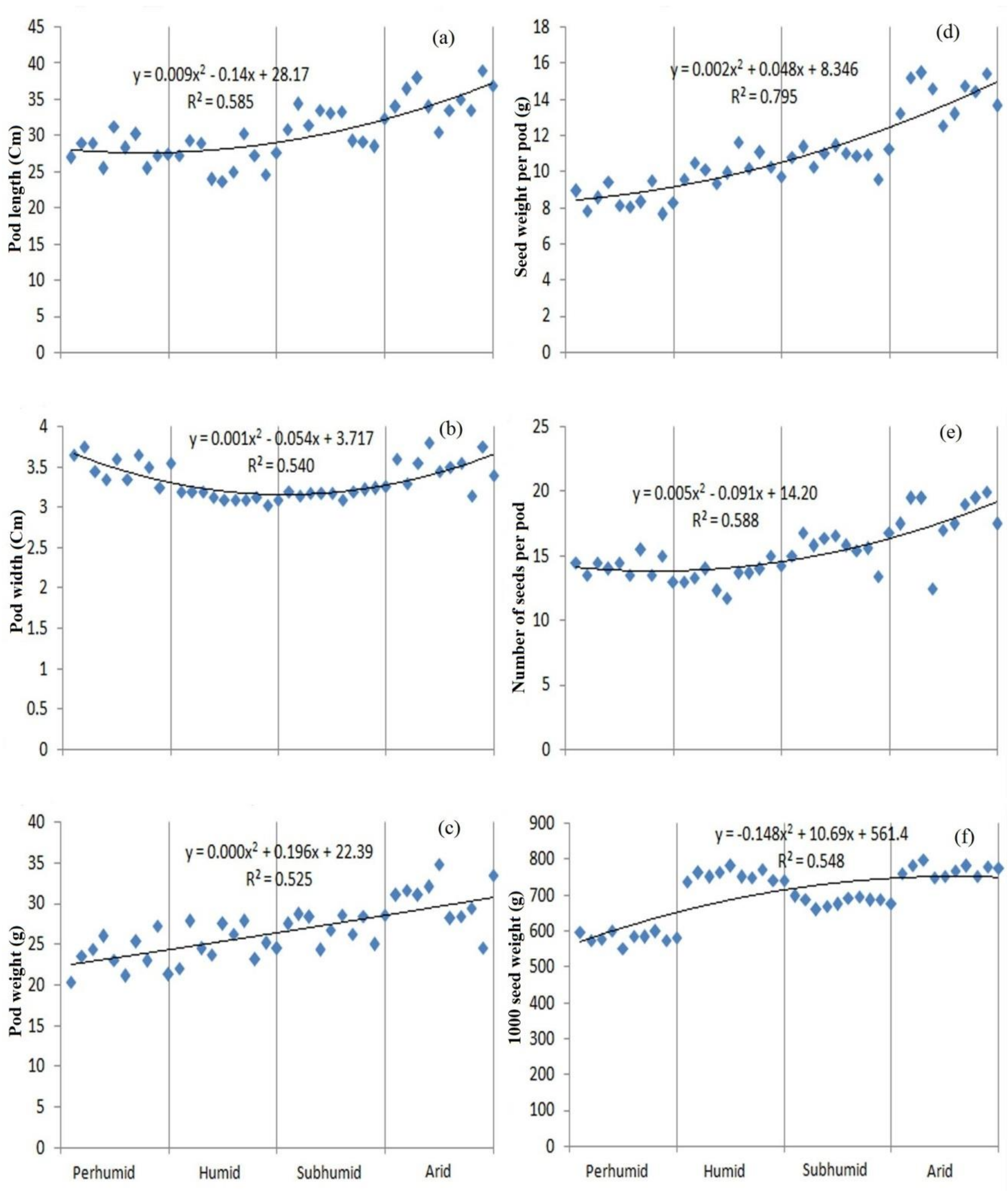

Figure 7.3. Effect of agro climatic zones on the pod and seed characters of *P. timoriana*

7.4.3 Effect of agroclimatic zone on seed germination and seedling growth

Another important parameter use by tree breeder for improvement programme is germinability. Total germination of *P. timoriana* seeds was found to be maximum in seeds collected from the arid region (64.58%), while least was found in seeds from perhumid region

(36.46%). Similarly, the overall speed of germination and its energy was maximum in arid and minimum in perhumid zone (Table 7.3). All seeds from the four zones took 5 days for the initiation of germination with shortest mean germination time in humid (10.9 days) while perhumid took the longest time (13.47 days). Cumulative germination curve (Figure 7.4) of the different zones showed that germination time of seeds from humid was the shortest (16 days), followed by subhumid and arid (23 days) and finally perhumid (27days). In this study, the value of germinability, germination energy and germination index for the four zones were observed as Arid > Humid > Subhumid > Perhumid. A similar trend was seen (Fig. 7.3 f) in the regression line drawn between seed weight and agro-climatic zone. Hence, we could deduce that arid, humid and subhumid zones corresponding to heavier seed weight resulted in giving high germination percentage. A strong positive relation between seed weight and germinability was already reported for this species (Thangjam and Sahoo, 2016). However, role of other unseen hereditary genes could not be ruled out in causing variability in germination.

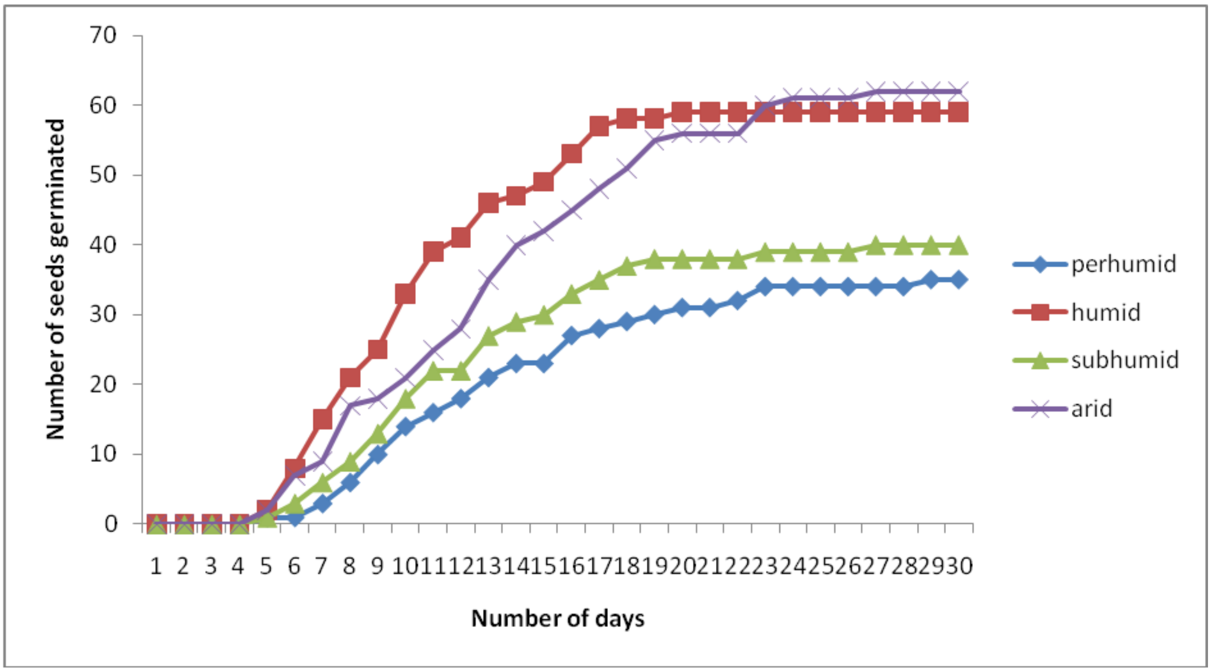

Figure 7.4. Relationship between zonal distribution of *P. timoriana* and its germination time

Table 7.3. Germination behaviour of the seeds of *P. timoriana* from different agro-climatic zones

Zone	Number of seeds sown	Germination %	Number of days for initiation of germination	Mean germination time (days)	Germination Index	Germination Energy
Perhumid	96	36.46±3.12	5±2	13.47±1.53	13.86±1.42	29.17±0.01
Humid	96	61.46±16.9	5±1	10.9±0.74	21.01±8.28	49.63±13.81
Subhumid	96	41.67±11.45	5±0.8	12.02±0.66	15.51±6.41	32.92±8.1
Arid	96	64.58±4.16	5±0.5	13.19±0.5	24.54±2.86	56.25±4.17
LSD<0.05		16.27		2.73	8.31	13.12

Table 7.4. Relationship between seedling length, biomass and collar diameter after 90 days of growth for different climatic zones

Zone	Seedling length (sdl)	Biomass (bms)	Collar diameter (cd)	Regression equation	r^2 (%)	F(α=0.05)
Perhumid	57.5±2.72	2.30±0.07	4.47±0.06	sdl=185.61+65.62bms-62.44cd	28	0.61
Humid	55.5±2.04	2.29±0.12	4.44±0.28	sdl=4.83+25.49bms-1.77cd	96	7.96E-05
Subhumid	60.8±2.14	2.65±0.14	5.05±0.12	sdl=44.58+14.08bms-4.18cd	54	0.0099
Arid	66.92±4.66	2.45±0.18	4.75±0.21	sdl=22.05cd-2.29bms-32.23	88	0.042

After 90 days from the two-leaf stage, seedling growth parameters such as seedling length, biomass and collar diameter were measured and a regression equation was derived for every agro-climatic zone. Multiple regression analysis between seedling length, biomass and collar diameter showed significant positive relationship ($P<0.05$) for humid, subhumid and arid zone. Similarly the coefficient of determination of the above parameters revealed that these three zones have more than 50% values that fit the model (Table 7.4). Perhumid zone, on the other hand, gave poor determination coefficient (R^2=28%) along with non-significant F value (0.61).

Figure 7.5, showed the effect of zonal distribution on the rate of growth of the seedling (RGR), average growth rate (AGR) and shoot to root ratio. Here, RGR was seen highest for seeds from the sub-humid zone (0.232g) while lowest for seeds from arid zone (0.179g). Average growth rate (AGR) after 90 days was maximum for seeds from the sub-humid zone (0.396g), while minimum in per humid seeds (0.299g). On the contrary, ratio of root to shoot in biomass production revealed that seeds from perhumid zone accounted to maximum difference (2:6.19) while minimum was found in arid zone (2:5.30).

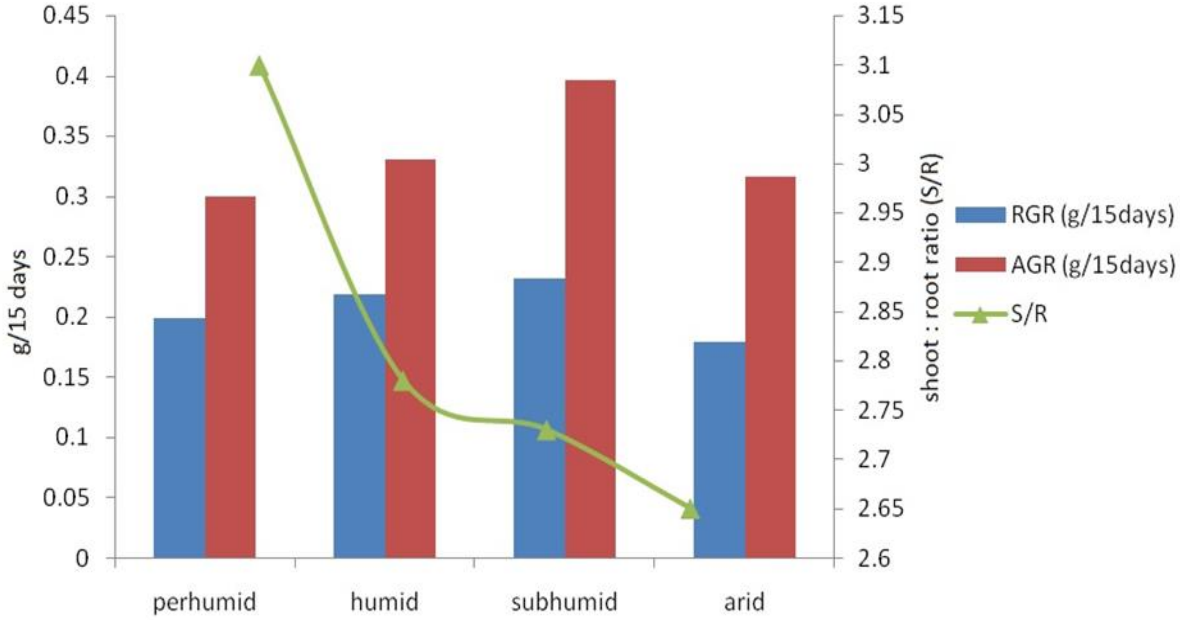

Figure 7.5. Effect of Zonal distribution on the relative growth rate (RGR), average growth rate (AGR) and shoot to root ratio (S/R) of *P. timoriana*

Simple regression curve drawn between seedling vigour and number of days (Figure 7.6) showed a distinct growth line in the average growth whose observed values are least deviated from the mean (R^2= 0.995). Seeds drawn from arid zone gave the maximum seedling vigour while perhumid zone resulted in minimum seedling vigour. Therefore using these equations one could predict a dependent value by knowing the other two independent values. Along with germination parameters, above seedling growth parameters also correspond to a high value in case of arid and sub-humid zones. Our results go hand in hand with the findings of other workers who acknowledge that seed size and weight have a strong influence on germination as well as growth and biomass of a plant (Oni and Bada, 1992; Souza and Fagundes, 2014; Kolodzijek, 2017). On the other hand, since these seedlings were raised under common nursery condition the environmental influences were reduced to minimal, hence, we could assume that the resultant variation in germination and growth is genetic in nature (Sniezko and Stewart, 1986).

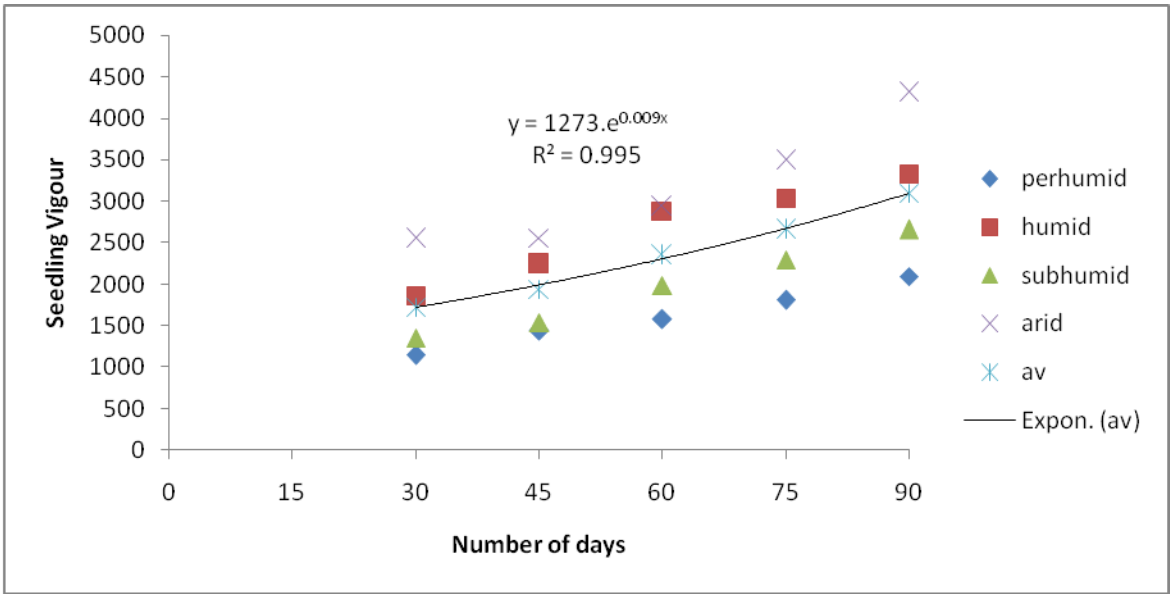

Figure 7.6. Relationship between seedling vigour, zonal distribution and time in days, taken alternately after every 15 days interval

From the overall result, we could conclude by accepting the hypothesis that there is significant variation among population in seed, pod and seedling traits with respect to geographic and ecological clines.

CHAPTER 8

Developing stem volume equation for

Parkia timoriana (D.C.) Merr.

8.1 Introduction

Volume and volume equation plays a pivotal role in silviculture, forest mensuration, management and in the estimation of carbon stocks and carbon sequestration potential of trees. Every tree has a particular structure that can be represented by its diameter, height and form. Hence, a particular volume equation may be a good fit for one species but not to another. On the other hand, the same species in different geographical areas may give slightly different increments, tree form and branching pattern. Therefore, there is a need for constant upgrading and/or development of new volume equation that fits diverse geographical range. For example, work done on *Dalbergia sissoo* (Khan and Faruque, 2010),*Pinus spp* (Lee et al., 2014, 2017), and *Pinus thunbergii* (Park et al., 2015) have shown constant improvement on the already established equations while work on *Aquilaria malaccensis* (Islam and Chowdhury, 2017) and *Eucaluptus* hybrid (Tewari and Singh, 2006) developed new volume equation. Nevertheless, a critical review on model development, common mistakes and corrective measures have been discussed and use of fewer explanatory variables has been recommended for ease model application and validation (Sileshi, 2014, 2015).

In India, Forest Survey of India (FSI) has been working to generate volume (growing stock) of various trees since 1965 when it was first called Pre-investment Survey of Forest Resources (PISFR). Since then various workers have developed volume equations for different fast-growing and economically important trees in different parts of the country. *Parkia timoriana* (Tree beans, Mimosoidae) is mainly concentrated in parts of eastern Himalayas of India though found in other South-East Asian countries too. It served as an important food supplement with varied uses which led to its dominance in home gardens and shifting agriculture lands of these regions (Sahoo, 2009). Shifting cultivation (locally known as ‘jhum’) is a type of traditional land use system in which a vast area of land is burnt, cultivated for 2 to 3 years and then shifted to another patch of land to repeat the same process. In spite of immense economic importance, this tree has a lack of valuation because of insufficient knowledge on volume and biomass. Few workers have determined growth equation and biomass of the species up to seedling stage (Thangjam and Sahoo, 2016) but no work has been done so far for older trees. This may be because destructive method of estimating volume is difficult for this species as most of the stakeholder of *Parkia* trees are local tribals whose livelihood depend on the income generated by selling the fruits (pods). Hence experimental set up for volume estimation for this species could only be performed through allometric and non-destructive means which consequently make our study important.

This paper aims to generate the best fit volume model of *Parkia timoriana* by adopting non-destructive process with diameter at breast height and height as independent variables, which could further be used to estimate biomass, carbon stock and carbon sequestration in the near future. Determining biomass and carbon stock is important as this will give an estimate of the amount of carbon that has been sequestered or emit when per unit of the tree biomass is formed or destroyed. Moreover, since this tree occupies a valuable space among the home gardens and jhums of these regions (Sahoo and Rocky, 2015), we could assume a larger contribution of it towards carbon assimilation and which could only be proved by further assessment of biomass and carbon stock. This study could also be used as a reference for other *Parkia* species found in subtropical home gardens as the attempt is first in the genus.

8.2 Materials and methods

8.2.1 Study area

Twelve populations representing four states of northeast India were thoroughly surveyed based on the availability of *P. timoriana* with different diameter class (Figure 8.1 and Table 8.1). The sampling sites represented different topographic and climatic variations with altitude ranging from 60 (Jiribam) to 1428.5 meters above sea level (Shillong), average annual temperature of 17.4˚C in Shillong to 28.6˚C in Jiribam, and annual rainfall of 118 cm in Medziphema to 353cm in Shillong. Variation in the study area is taken to minimise the error and broaden the acceptance of the resultant model.

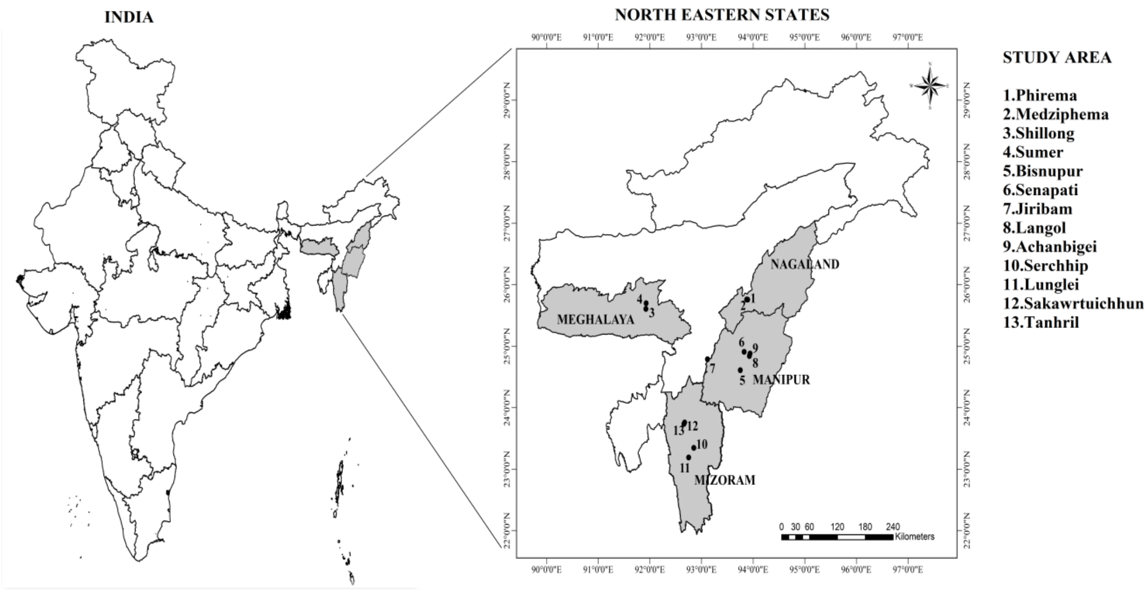

Figure 8.1. Study area for volume estimation and validation

8.2.2 Study species

Parkia timoriana is a multipurpose agroforestry tree species that occupy the overstorey of home gardens and shifting agriculture lands in Eastern Himalayan foothills. In this region, the plant provides a good source of livelihood for both hill and valley people during winter to early spring season. Pods are sold at high price fetching a market value ranging from Rs. 70 to 120 Kg^{-1} (Firake et al., 2013). The pods and seeds are important seasonal food and also a good source of protein (Mohan and Janardhan, 1993) and antioxidant (Tapan, 2011); bark, seed and pod are used as a good and effective medicine for curing various ailments (Samuel et al., 2010; Ong et al., 2011; Rathi et al., 2012), while branch and stem are used as firewood (Rocky and Sahoo, 2004). Due to its multiple uses, *P. timoriana* is highly favoured by every household which explains its dominance in home gardens and jhum lands and ultimately makes it important for us to study its volume, biomass and carbon sequestration potential.

8.2.3. Data collection for the best fit model

Bole of *Parkia timoriana* is nearly circular, therefore measurement was taken by using measuring tape as it gives more reliable result than a calliper. For determining the diameter class, a random sampling was done on population 12 by taking 30 trees to identify the range of diameter at breast height (DBH). The 12^{th} population was chosen according to our convenience. At approximately 33^{rd} percentile the 30 trees were divided into three classed namely: DBH≤0.1m, DBH = 0.1 to 0.2and DBH ≥ 0.2. These diameter classes were used to select trees for the remaining 11 population. Thus, each population is represented by 30 trees; three diameter classes with 10 trees each (Table 8.1).

Table 8.1. Summary statistics of sampled *P. timoriana* trees

Diameter class (m)	Statistics	DBH (m)	Height (m)	Volume (m^3)	No. of trees
<= 0.1	mean	0.0787	6.50	0.07336	120
	SD	0.0145	0.52	0.03816	
	Min	0.0432	5.00	0.00080	
	Max	0.0998	9.50	0.16649	
0.1-0.2	mean	0.1563	9.80	0.29480	120
	SD	0.0257	2.57	0.12895	
	Min	0.1003	5.30	0.09305	
	Max	0.2000	16.00	0.71105	
>0.2	mean	0.2515	12.69	0.90367	120
	SD	0.0387	1.73	0.42299	
	Min	0.2006	9.00	0.31583	
	Max	0.4500	17.00	3.44250	

8.2.4 Data collection for validation of the best fit model

Forty trees irrespective of diameter class or normality distribution were chosen randomly from the 13^{th} population to validate the best fit model.

8.2.5 Volume estimation

Parkia tree has straight bole with a good number of branches on top. Figure 8.2 gives a brief description of the branching and the tree form that is assumed for the study. The number 1 to 8 mentioned in the figure indicates the places where the diameter is measured. Measurement 3 to 6 were done by climbing the tree while 7 and 8 were measured by placing a self-designed "30˚ angle scale" placing just above the branch where the diameter needs to be taken. The angle scale was then zoomed in by using a 60 X zoom Nikon P600 Coolpix camera and the readings were noted. The reading, when multiplied by twice the value of tangent 30˚ (0.577), gave diameter of the measures region of tree branch.

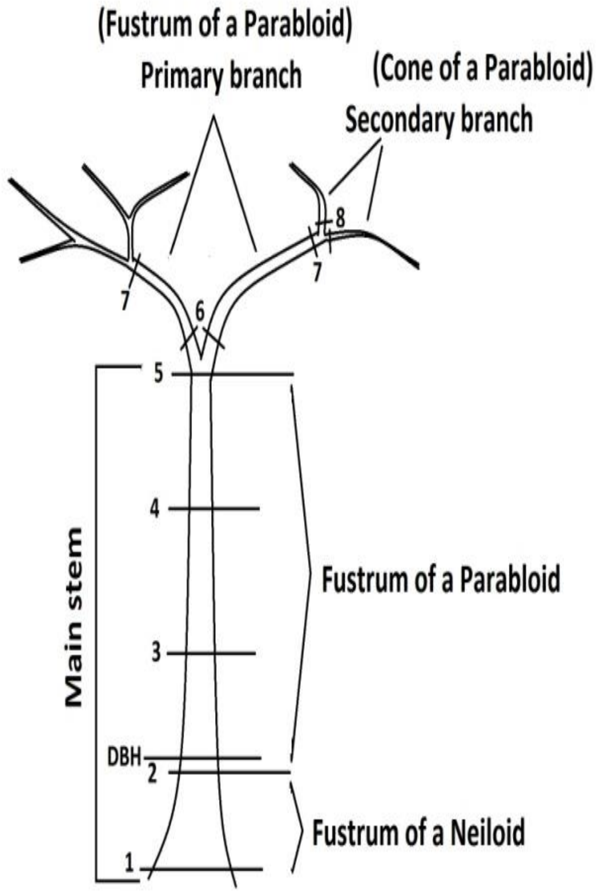

Figure 8.2. Branching pattern and estimation of diameters in a different section of a *P. timoriana* tree.

8.2.6 Volume equation and computation

Parkia timoriana tree as a whole, as shown in figure 8.2, could be divided into three separate forms each having different volume equations. The bottom part of the stem above stump height was considered a frustum of neiloid for which the volume (V) was calculated as V= $\frac{\pi H}{12} \times (D^2 + D \times d + D^2)$. where, D is the basal diameter, d is the top end diameter and H is the height of the frustum. The next part of the tree is frustum of paraboloid which includes the remaining part of the stem and the main branch(s). Here, the cross-sectional area was measured at 2,3,4,5, 6 and 7. The length from 2 to 5 has equal intervals that depend upon the height of the tree. Therefore four volumes for the frustum of paraboloid were calculated by using Smalian's formula as V= $\frac{C+c}{2} \times L$. Where C is the cross-sectional area at the larger end, c is the cross-sectional area at the smaller end and L is the length of each section. Secondary branches of tree were considered as cone and hence volume was calculated as V= $\frac{1}{3} \times b \times h$. Where b is the basal area and h is the length of secondary branch. The overall volume thus obtained is the actual volume of the tree. Above procedure is followed in all 360 trees for calculating actual volume per tree. The volume thus obtained was then subjected to model fitting and validation for finding the best fit model.

8.2.7 Model computation and fitting statistics

Altogether 19 volume equations (Table 8.2) which were previously used by other scientists (Bi and Hamilton, 1998; Tewari and Kumar, 2001; Islam and Chowdhury, 2017) were developed and compared in this study by using Linear mixed model (LMM). In this process, we used Population as the random variable while diameter (D) and height (H) as a fixed variable. Based on the number of independent variables, these models were grouped into 2: volume equation with one independent variable (D) and volume equation with D and H as independent variables.

Table 8.2. Local and general volume equations using diameter and height.

Type of volume equation	Model no.	Equation	Number of co-efficient
Local (D)	1	$V = a + bD$	2
	2	$V = a + bD + cD^2$	3
	3	$V = aD + bD^2$	2
	4	$V = a + bD^2$	2
	5	$V = a + b\ln D$	2
	6	$\ln V = a + b\ln D$	2
	7	$\ln V = a + bD$	2
General (D & H)	8	$V = a + bD^2\mathrm{H}$	2
	9	$V = a + bD + cH$	3
	10	$V = a + bD + cDH$	3
	11	$V = a + bD + cD^2\mathrm{H}$	3
	12	$V = a + bD + cH + dDH$	4
	13	$V = a + bD + cH + dD^2\mathrm{H}$	4
	14	$V = a + bD^2 + cH + dD\mathrm{H}$	4
	15	$V = a + bD^2 + cH + dD^2\mathrm{H}$	4
	16	$V = a + bD^2 + cDH + dD^2\mathrm{H}$	4
	17	$\ln V = a + b\ln D + c\ln H$	3
	18	$V = a + b\ln D + c\ln H$	3
	19	$V = a + bD^{-1} + cH^{-1}$	3

These models were verified for the best fit by calculating coefficient of determination(r^2), root mean square error (RMSE), mean deviation (MD), mean absolute deviation (MAD) and Akaike's Information Criterion (AIC) following similar works on other tree species (Lumbers et al., 2013; Lee et al., 2014; Park et al., 2015). The following equations were used for the above test of models:

$$r^2 = 1 - \left[\sum_{i=1}^{n}(V_i - \hat{V}_i)^2 / \sum_{i=1}^{n}(V_i - \bar{V})^2\right]$$

$$RMSE = \sqrt{\sum_{i=1}^{n}(V_i - \hat{V}_i)^2 / n}$$

$$MD = \sum_{i=1}^{n} (V_i - \widehat{V}_i)/n$$

$$MAD = \sum_{i=1}^{n} |V_i - \widehat{V}_i|/n$$

$$AIC = n \times \ln(MSE) + 2P$$

Where, V_i= measured volume of the ith tree, $\widehat{V}_i$= predicted volume of the ith tree, $\bar{V}$= measured mean tree volume, n= the total number of trees, MSE = mean square of error, P= number of parameters used in the model.

Ranking (Rk) was given to all the resultant values of r^2, RMSE, MAD and AIC and the final ranking was estimated by ranking the aggregate rank of the models. Lesser the value of RMSE, MAD and AIC with r^2 closer to 1 the more the model is fitted (Adequnle et al., 2013; Brahma et al., 2017).

8.2.8 Cross-validation of Models

All the 19 models were cross-validated following 'hold-out method' of SPSS (version 20). Out of 360 trees which were used for preparing the model 80% were randomly selected and analysed for linear regression. Using the coefficients, data transformation was done to give a predicted volume for every model. These predicted volumes were then compared with the actual volumes by splitting the data and followed by bivariate correlation and generalized linear modelling. The resultant value of correlation (r), regression (r^2), root mean square error (RMSE), AIC and Bayesian information criterion (BIC) were used to cross-validate the models.

8.2.9 Validation with an extra population

Models from the best sit statistics were validated by using data from a 13th population with 40 trees selected randomly from Tanhril area of Mizoram state (23.7372˚N & 92.6606˚E). Paired t-test, correlation coefficient (r) and coefficient of determination (r^2) were analysed to see the degree of similarity. Regression line and residual plots were also drawn to see the interrelation between the predicted volume and the actual volume of the extra population.

8.3 Statistical analysis

Microsoft Excel 2007 and IBM SPSS 20 software was used for the Analysis of variance (ANOVA), Linear mixed-effects model (LMM) and generalized linear modelling (GLM) of the sample trees.

8.4 Results

8.4.1 Coefficient estimation and selection of best fit model

The coefficients and model fit statistics using LMM for 19 models are presented in table 8.3 and 8.4. The final ranking of all the models using r^2, RMSE, MAD and AIC as the base parameters have shown that volume derived from model 8 gave the best fit. The overall ranking from best to worst was found as 8>14>2>3=12>4>17>6>9=1>7>5. However after division, the ranking for models of V= f(D) was found as: 2>3>4>6>1>7>5, and for V= f(D,H) as: 8>14>12>17>9>19. All coefficients (a,b,c and d) are valid with P<0.001 for models with D as the single independent variable, while only 6 out of the 12 models showed significant coefficients. Coefficient (b) of model 10; (a) and (b) of model 11; (a), (b) and (c) of model 13; (b) and (c) of model 15 and 16; and (a) of model 18, were not significant as

P>0.05. Therefore, models 10, 11, 13, 15, 16 and 18 were not valid for ranking under fitting statistics.

Table 8.3. Coefficients for 19 volume models of *Parkia timoriana.*

Model No.	Coefficients				Standard Error			
	a	b	c	d	a	b	c	d
1	-0.42062**	5.20775**			0.0237	0.1198		
2	0.12500**	-2.40265**	21.43248**		0.0267	0.3148	0.8632	
3	-1.13538**	18.25535**			0.1553	0.5309		
4	-0.05795**	15.02068**			0.0124	0.2137		
5	1.77941**	1.60808**			0.0517	0.0580		
6	1.35898**	2.36439**			0.0345	0.0380		
7	-1.70607**	6.61061**			0.0285	0.1539		
8	0.03177**	1.03200**			0.0054	0.0096		
9	-0.49110**	4.62036**	0.01715**		0.0319	0.2290	0.0056	
10	-0.12149**	-0.54662NS	0.35873**		0.0229	0.3290	0.0197	
11	0.02485 NS	0.07107NS	1.02089**		0.0147	0.1399	0.0257	
12	0.55240**	-2.96556**	-0.09354**	0.71074**	0.0322	0.2317	0.0040	0.0195
13	0.03190 NS	0.10363NS	-0.00140 NS	1.02407**	0.0192	0.1513	0.0025	0.0263
14	0.37949**	-4.56265*	-0.09842**	0.64604**	0.0486	1.5730	0.0097	0.0540
15	0.04236*	-0.60073 NS	-0.00087 NS	1.07795 **	0.0186	0.7281	0.0020	0.0517
16	0.02933*	-0.80484 NS	0.01276 NS	1.04805 **	0.0102	0.7624	0.0135	0.0547
17	0.67134**	2.09985**	0.48264**		0.1833	0.0790	0.1264	
18	0.38258 NS	1.07201**	0.98157**		0.2679	0.1161	0.1847	
19	1.42714**	-0.01697*	-7.57512**		0.0566	0.0068	0.8264	

**=significant at P<0.001, *=significant at P<0.05 and NS=not significant

Table 8.4. Model fit statistics for volume equation of *P.timoriana.*

Model no.	Adjusted r^2	Rk	RMSE	Rk	MD	MAD	Rk	AIC	Rk	∑Rk	Final Rk
D											
1	0.837	5	0.176068	4	5.60E-03	0.126742	5	-223.568	5	19	5
2	**0.939**	**1**	**0.104881**	**1**	**6.06E-07**	**0.072569**	**1**	**-583.693**	**1**	**4**	**1**
3	0.936	2	0.109545	2	0.022409	0.082791	3	-567.932	2	9	2
4	0.929	3	0.114018	3	3.27E-07	0.082518	2	-530.061	3	11	3
5	0.678	7	0.246982	5	6.42E-07	0.170887	7	24.303	7	26	7
6	0.912	4	0.164317	6	9.51E-03	0.117970	4	-276.002	4	18	4
7	0.836	6	0.223607	7	9.51E-03	0.151612	6	-48.150	6	25	6
D&H											
8	**0.970**	**1**	**0.077460**	**1**	**1.07E-07**	**0.050443**	**1**	**-820.562**	**1**	**4**	**1**
9	0.842	5	0.173205	5	3.93E-06	0.128232	4	-223.144	5	19	5
10	0.915		0.126491		-3.89E-07	0.089696		-448.040			-
11	0.970		0.077460		-3.41E-07	0.050643		-818.723			-
12	0.931	3	0.114018	3	-9.62E-01	0.961874	5	-770.147	2	13	3
13	0.970		0.077460		1.28E-06	0.050419		-808.876			-
14	0.952	2	0.094868	2	-1.84E-06	0.065742	2	-650.660	3	9	2
15	0.970		0.077460		3.99E-06	0.049870		-812.230			-
16	0.970		0.077460		-8.39E-07	0.050257		-816.528			-
17	0.917	4	0.158114	4	9.51E-03	0.109595	3	-286.181	4	15	4
18	0.702		0.236643		5.48E-07	0.166345		0.587			-
19	0.546	6	0.293258	6	-1.74E+00	1.745519	6	114.788	6	24	6

8.4.2 Cross-validation of the models

An equation without validity is vague. Therefore, all the 13 equations which were found valid under LMM were cross-validated using hold-out method by splitting the data randomly into 80% and 20 %. Table 8.5 shows the values r, r^2, RMSE, AIC and BIC of the split data when compared with the original volume. All the values r and r^2 showed significant at $P<0.001$ with most of the models fitting 80% gave a larger correlation and determination than the corresponding 20% data fitted models. The overall ranking of RMSE, AIC and BIC for the 13 models also resulted 'model 8' as the best fit while 'model 5' as the least. For models with

V= f(D) ranking from best to worst was given as: 2>3>4>6>1>7>5 and for V= f(D,H): 8>12>14>17>9. Values of cross-validation of model 19 were not given in the table due to multicollinearity between the predictors.

Table 8.5. Cross-validation of the models using hold-out method

Model	r		r^2		RMSE		AIC		BIC	
D	20%	80%	20%	80%	20%	80%	20%	80%	20%	80%
1	0.927	0.914	0.858	0.836	0.138	0.184	139.507	541.028	144.087	548.347
2	0.961	0.971	0.922	0.942	0.100	0.110	138.901	534.822	143.482	542.141
3	0.960	0.969	0.921	0.938	0.100	0.114	138.91	535.044	143.491	542.363
4	0.958	0.965	0.917	0.932	0.105	0.118	138.943	535.425	143.524	542.744
5	0.857	0.820	0.730	0.672	0.190	0.259	140.708	550.521	145.288	557.840
6	0.965	0.954	0.931	0.909	0.118	0.173	139.151	539.916	143.732	547.235
7	0.939	0.911	0.880	0.829	0.155	0.237	139.878	547.391	144.459	554.710
D&H										
8	0.975	0.987	0.950	0.973	0.084	0.071	138.633	533.026	143.213	540.345
9	0.927	0.917	0.850	0.843	0.141	0.179	139.582	540.561	144.163	547.880
12	0.972	0.985	0.945	0.971	0.084	0.077	138.686	533.168	143.267	540.487
14	0.964	0.978	0.928	0.957	0.095	0.095	138.839	533.967	143.420	541.286
17	0.971	0.955	0.942	0.912	0.110	0.170	138.993	539.636	143.574	546.955

8.4.3 Independent validation test

Resultant best-fit model i.e. model 2 and 8 were again validated with a 13^{th} population taken exclusively of the other 12 populations. Both models gave high r (0.916, 0.935) and r^2 (0.838, 0.875) values though lesser than the above 12 populations (Figure 8.3 and Table 8.5). Figure 8.3 also shows that the residuals are distributed randomly and have a constant spread throughout the range of fitted volume.

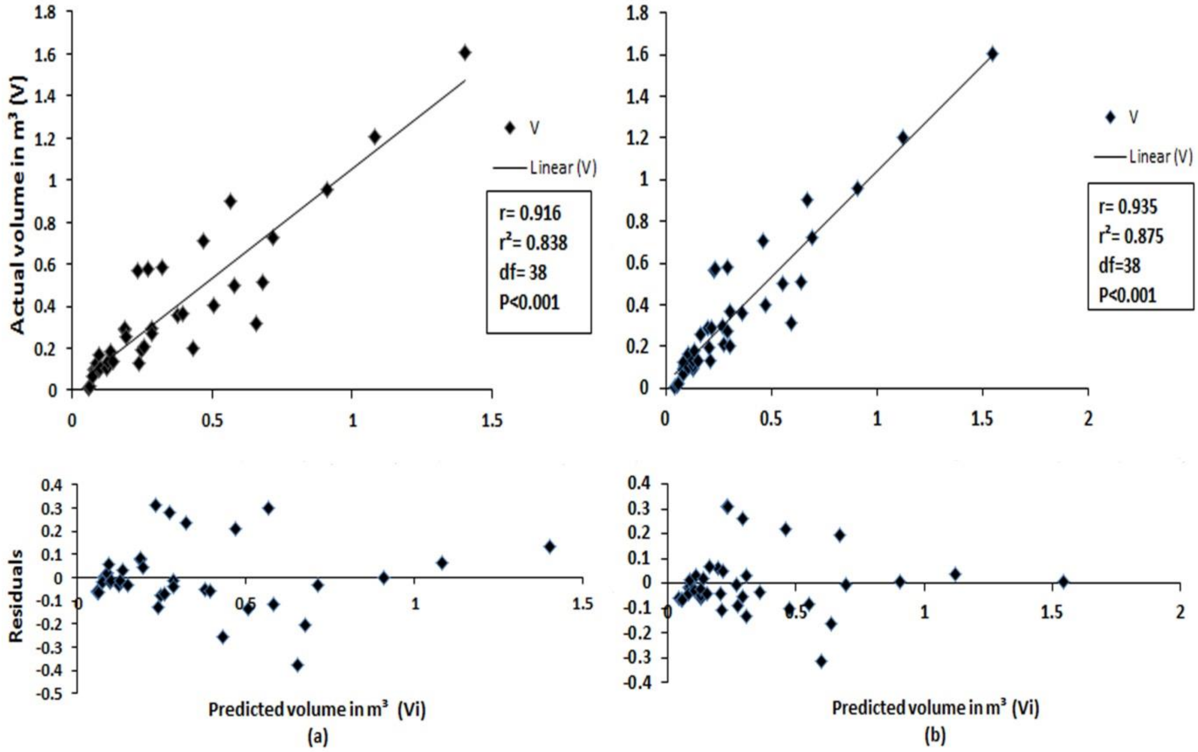

Figure 8.3. Regression and residual plot of the 13th population using (a) model 2 and (b) model 8.

Result of paired t-test of the observed and predicted volume for model 2 (P= 0.137, df= 38) and 8 (P= 0.063, df= 38) on population 13 has shown no significant difference. Here, single outlier each for model 2 and 8 were not considered in running the test.

8.5 Discussion

The results from our analysis could conclude that model 2 and 8 represents the best fit for volume estimation of *P. timoriana* with diameter at breast height ranges from 0.0432m to 0.45m. Similar result was obtained in studies done on *Aquilaria malaccensis* in Bangladesh (Islam and Chowdhury, 2017) which gave model 2 as the best fit, while studies on *Pinus taeda* (Burkhat, 1977; Sherrill et al., 2011), *Pinus densifolia*, *Pinus koraiensis* and *Larix kaempferi* (Lee et al., 2017) conclude model 8 as the best fit model. In addition, model 2 overestimates the volume with an MD of -1.06E-07m while model 8 underestimates by an

MD of 1.07E-07m. A (-) MD represents over prediction of a model and (+) MD represents an under-prediction (Lumbers et al., 2013). Comparison between these two models, $V=a + bD^2H$ gave higher accuracy with 0.02 MAD difference, and if this is acceptable, $V = aD + bD^2$ using D is valid enough to be used in the field considering the difficulties of measuring the tree height in hilly terrain.

In this paper, we used LMM over conventional model as many workers have identified the superiority of it to give a more stable equation (Sileshi, 2015; Nath et al., 2019). Unlike conventional models, LMM uses random effect and ignoring it might lead to underestimation of accuracy. Working of LMM uses a likelihood function, a specified probability for parameters estimated and Maximum Likelihood Estimation method to maximize the accuracy by fiddling around with the parameters. Further study on the limiting behaviour of empirical BLUPs (best linear unbiased predictors) and BLUEs (best linear unbiased estimates) showed the benefit of using LMM in smaller data set as it does not require a normal distribution of random variables (Jiang 1998).

Out of the 19 models, only 14 were selected by using LMM and 13 by hold-out cross-validation process due to the presence of multicollinearity between the predictor variables. Multicollinearity is said to be present when a change in value of one variable resulted in the dramatic change in regression coefficients of the previous variable (Yoo et al., 2014). Cross-validation was done to check the extent to which the volume prediction holds with minimal bias. The advantage of cross-validation is that both training and validation used all data points, and each data point was used for validation exactly once (Refaeilzadeh et al., 2009). Therefore cross-validation in predicting stem volume equation of *P. timoriana* was necessary so that the appositeness of the volume equation remains for other independent datasets in different home gardens and shifting cultivation areas of northeast India. However, since hold-out validation has chance of skewness of the result (Refaeilzadeh et al., 2009) the best fitting models were again validated with an independent sample data (13^{th} population).

Validation with the 13^{th} population is important to check the applicability of the proposed model to other geographic ranges. Both models 2 and 8 gave high r and r^2 value but lesser than those observed in the cross-validation of the 12 populations. This was expected as any population different from the sampled 12 populations have a greater chance of deviation than among populations. Only regression analysis is not sufficient to validate a model, checking the residual plot or observed error decides the validity of the model regression. For a valid model the residuals should not be either systematically high or low and should be centralized on zero throughout the range. Our results satisfy the above criteria along with high value of correlation, determination and non-significant paired t-test. Therefore, the test model 2 and 8 are valid to reproduce for other geographic range.

In addition, the scattered plot drawn between predicted volume and observed volume of the 13^{th} population (Figure 8.3) could also conclude that *Parkia timoriana* trees of lesser diameter have less deviation than larger ones. Similar observations were found in *Pinus densifolia*, *Pinus koraiensis* and *Larix kaempferi* by Korean Forest Research Institute in South Korea (Lee et al., 2017).

CHAPTER 9

Conclusion and recommendation

Effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana* (DC.) Merr.

It is evident from the results of the present study that seed, pod and seedling parameters vary considerably between provenances of *P. timoriana*. In this study rather than selecting the best provenance, we tried to select the best traits by comparing their performance on both among and between provenances. Therefore, if the objective of selection of seed is to get higher pod characters such as pod length, pod width, seed weight per pod and seed number per pod, then procurement of seeds should be made from low winter rainfall areas. Heavier seeds and pods are also associated with low latitude areas. Therefore the selection of the species for these traits should be made from equatorial regions. Seeds from lower altitude tend to give larger seedling collar diameter. However, if the selection has to be done from the studied provenances then the best provenance for the respective traits should be taken as the seed source. Among the traits, those having high (h^2) value could be used for improvement purpose as high heritable traits have the least chance of deviation due to the environment. Therefore, the characters such as germination percentage, germination energy and seedling collar diameter could be used for further research related to tree improvement programme of *P. timoriana*.

From the study, it could be concluded that selection of the improved genotype, would be useful to create stress-tolerant and better quality trees which would primarily help Parkia dependent people of North-east India in sustaining their livelihood.

The present work will give more extensive result if assessment continues throughout different stages of growth of the plant. Further research is recommended on multi-site trial and the use of genetic marker to get more accurate data at genetic level. It is also suggested to explore the possibility of crossing over among different species as some authors have mentioned that *P. intermedia* as a hybrid developed long ago between *P. speciosa* and *P. timoriana*. If this is possible then higher level of improvement for desired traits could be done, which may give even better result than within species hybridisation.

Seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* (DC.) Merr. in North-east India

We present strong evidence that the agroclimatic zones of *Parkia timoriana* are associated with difference in seed, pod and regeneration characters. It was also found that different populations under similar agro climate gave similar response toward growth and regeneration. This indicates that the gene pool of *P. timoriana* varies largely with change in climatic condition. Interestingly, the performance of these zones in terms of germination and vigour followed a parallel trend with that of the weights of seed and pod; Arid > Humid > Subhumid > Perhumid. Therefore, higher aridity reflects heavier seeds and in turn reflects better germination and vigour.

Therefore, we should confer and refer the breeders to choose seeds from drier/arid zone in establishing a future seed orchard for distribution to villagers of other agroclimatic zones or any other clients on demand. On the other hand, moe number of sample sites is suggested for spatial distribution of agroclimatic zone by using geospatial tools as there was slight variation in our study when compared to the data established from moisture index by following Thornthwaith model. With this one might able to draw a precise model with map that depicts seed characters of *P. timoriana* by giving the moisture index or just by giving the location point.

Developing stem volume equation for *Parkia timoriana* (DC.) Merr.

Development of species-specific volume equation is necessary since there is no universal volume equation that is applicable to all species. Our present study focused on estimating and analysing the best volume model(s) of *P. timoriana* without destructive process which is essential as it is cost-effective and also save time and unnecessary sacrifice of many economically important trees. These superior models can be applied for use in the determination of above-ground biomass, carbon stock and carbon sequestration potential at multiple sites both at local and regional level.

Further, studies may use harvest data from research plantations inside Mizoram University, Aizawl, India, to create biomass conversion factor for estimation of carbon stock. Similar works on species-specific modelling in northeastern states of India is recommended as the region harbour huge stock of plants without proper volume and biomass assessment.

Effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana*

Both physical (exogenous) and physiological (endogenous) inhibition are likely to be the cause of dormancy in the seeds of *P. timoriana*. Our results showed more positive skewed towards physiological dormancy, as tap water, GA_3 and cold treatment (stratification) gave a better result. Acid scarification, boiling water and nicking little affect the germination and initial growth parameters.

It is recommended that farmers and horticulturist, who are interested in plantation of *P. timoriana*, should be advised to use simple tap water before sowing. Other factors were also found to increase germination compared to the untreated seeds, but since water is freely available choosing other options is insignificant. We also recommend using fungicide especially for waterborne and seed-borne fungus along with the pre-treatments, as seed rot and damping-off of seedling are common (personal observation) in this species. Further work on priming of seeds of this species could be beneficial for large scale plantations that need higher germination in a very little time.

Effect of seed source and seed size/mass on seed germination and seedling growth

The effect of seed source on germination and seedling growth were already discussed in the first objective so the experiment is not reviewed here. Significant effects of different seed weight category on germination and growth were observed in our study. It was found that light and intermediate weight seeds of *Parkia timoriana* germinate faster than the heavy one. However, heavy seeds have the advantage of higher germination percentage and seedling vigour.

Variation in seed mass within species is associated with differential seed performance among habitats. In open disturbed areas lighter seeds of *P. timoriana* are more desirable as it gave faster germination to balance with the rapid changing habitat. In contrast, larger seeds have more reserves, producing vigorous seedlings that present greater competitive ability in forest understorey or fallow areas of shifting cultivation. This variation in seed size allows the species to colonize different habitats, helping to justify *P. timoriana's* widespread geographic distribution. To farmers and common people of Northeast India, higher germinability and seedling vigour are more important than speed of germination. Therefore, it is recommended for them to use heavier seeds to get the desired result.

During the study, it was found among *P. timoriana* tree growers that they prefer to take the middle and bottom seeds for plantation. Therefore, further work related to the effects of age of the tree and location of seeds on the pod, on germination and growth are advocated.

SUMMARY

Provenance represents the original native place where the seeds are sourced. Provenance trial focuses on selecting the best performing provenance by comparing its characters, or it can also be used for selecting the best characters by comparing the provenance. Both of which could be used in tree improvement programme. Effect of seed source variation and provenance trials are common area of research for herbs and shrubs but are rare for trees.

P. timoriana is an important agroforestry tree species, which is widely grown in home gardens and shifting agriculture lands of Northeast India. The plant provides a good source of livelihood for both hill and valley people during winter and early spring season. Pods and seeds are taken as food and are sold at high price fetching a market value ranging from Rs. 70 to 120 Kg^{-1} (Firake et al., 2013). Other important uses of the tree include antioxidant property, ethnomedicinal and cosmetic uses, and firewood supply for the energy-deficient hill people (Angami et al., 2018). Along with its wide uses, there is an urgent need of research on adaptive potential and regeneration ecology as report on dying of this tree is increasing year after year. Insect pests such as *Cadra cautella Walker* (Thangjam, 2016), fungus and tree borers damage heartwood, pod and seeds causing dieback of the tree.

Adaptive potential of every tree is responsible for the change in character, which in turn is supplemented by difference in genotypes, environments and their interactions (Savolainen et al., 2011). In forest and plantations of Asia-pacific region *P. timoriana* has shown variations in its morphometric characters. This variation maybe because of the result of evolutionary history characteristics, mating system, population density and gene flow mechanism (Hamrick, 2004). *P. timoriana* like many other species of mimosoidae have self-incompatibility. Pollen of this flower could not successfully fertilize with the ovule of the ovary directly without any external help. Hence, large gene flow is expected viz-a-viz genetic variation among the population. Moreover, natural habitat range of *P. timoriana* varies extensively from foothill to elevation up to 1300 m above sea level within northeast India (Hopkins, 1994) and thus, one may expect genetic divergence among its populations in several traits. Though there are reports on variations in some of the traits of *P. timoriana* in different regions, there is still lack of deeper scientific research on the individual traits representing individual characters for heredity and regeneration. Moreover, no studies have been made so far on the relation between provenance, maternal environment and germinability and seedling growth of the species.

Germinability and seedling growth are also affected by the type of dormancy and variation in seed weight. Normally, many trees exhibit seed dormancy so as to have a better chance of survival during unfavourable conditions. However, for plantation programme artificial favourable conditions are given to the seeds to overcome dormancy. Like many other leguminous trees, *P. timoriana* have hard-coated seeds. Under natural conditions, the seeds may take a much longer period to germinate thus necessitating use of pre-treatments (Aref et al., 2011) to increase the percent germination, rate of seed germination and finally seedling vigour. Seeds of this species also showed variations in mass and size. Seed mass represents a complex adaptive compromise (Harper, 1977). It plays a vital role in the establishment of the juvenile phase of a plant's growth curve. Different species give different results on the effect of seed mass on germination. Maximum work on this topic resulted in the more positive effect of larger seed mass, which might be because of the larger food reserve present. However, negative relationship between seed mass and relative growth rate were also reported in some species (Castro et al., 2008).

Another area of study of provenance is the effect of seed zones on germination and growth. Since *P. timoriana* has wide geographical distribution their provenance may represent distinct agroclimatic zone. Many workers have tried to find the relationship between seed source or seed zones on germination and growth both within species and between species on many species (Aigbe et al., 2016; Moya et al., 2017), however, very little quantitative estimates regarding the role of species' adaptation on different agro-climates is done on this genus and none on this species. An experiment is designed using climatic model of Thornthwaith (1948) climatic classification and further mapping with ArcGIS interpolation tool, which fit the provenance related climatic data of *P. timoriana* under various agroclimatic zones.

P. timoriana, being dominant species in home gardens and shifting cultivation lands in North-east India, it is assumed that the species is a potential carbon sink for this region. This research also tried to generate the best fit volume model of *Parkia timoriana* by adopting non-destructive process with diameter at breast height and height as independent variables, which could further be used to estimate biomass, carbon stock and carbon sequestration in the near future. Determining biomass and carbon stock is important as this will give an estimate of the amount of carbon that has been sequestered or emit when per unit of the tree biomass is formed or destroyed.

Assuming that variation in morphometric, germinative and seedling traits are influenced by genotype and environmental variation, we made an attempt to relate the available morphometric trait variations of this species with the geographic variations and seed source, and to identify the best seed source to counter the dying population of this species which could help to develop a cultivar having incorporated all its desired characters. Thus, the present work was carried out with the following objectives:

1) Effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana* (DC.) Merr.
2) Effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana* (DC.) Merr.
3) Effect of seed source and seed size/mass on seed germination and seedling growth.
4) Seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* in North-east India
5) Developing stem volume equation for *Parkia timoriana* (DC.) Merr.

The first objective of the thesis was about the effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana*. For this, 12 provenances representing 4 north-eastern states of India (Nagaland, Meghalaya, Manipur and Mizoram) were identified and selected for the present study for analysing the variation in different quantitative traits. The identified provenances were: (P1) Pherema, (P2) Medziphema, (P3) Shillong, (P4) Sumer, (P5) Bishnupur, (P6) Senapati, (P7) Jiribam, (P8) Langol, (P9) Achanbigei, (P10) Serchhip, (P11) Lunglei, and (P12) Sakawrtuichhun. Only mature trees of 12±2 years were taken for this experiment to maintain homogeneity in the result. Correlation coefficients were calculated for all possible geo-climatic traits, seed and pod related traits (SPT's), germinability and seedling growth traits. To draw an analogy between the magnitude of variation caused by genotype and environment, genotypic coefficient of variance (PCV) and environmental coefficient of variance (ECV) were estimated. Broad sense heritability (h^2) was also calculated to determine the percent of phenotypic variation contributing to the total variation. It was observed that morphometric, germinative and seedling parameters vary considerably between provenances of *P. timoriana*. Overall ranking of the performances found P1 as the best among seed traits, P7 as the best for pod traits, P12 as the best for germination percent and P10 as the best for seedling vigour. On the other hand effect of provenance on morphometric, germinative and seedling growth indicates the influence of geo-climate and other genetic factors on it. Estimates of genetic and environmental effects indicate that genetic coefficient of variance (PCV) was higher for germination percentage (GP), germination energy (GE) and collar diameter (CD). In all these characters environmental factors were found to play a minimal role as 75 to 95 % of the total variation was because of provenance variation as shown by the value of broad-sense heritability. Pod characters have significant negative correlation with winter rainfall. Heavier pods are associated with lower latitude areas. On the other hand, seed characters such as SWP and SWT were found increase with summer temperature. Seeds from lower altitude tend to give larger seedling collar diameter.

The second objective of the study was to determine the effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana*. Like many other legumes, *P.timoriana* also have hard-coated seeds which sought the need to investigate the most appropriate way to break its dormancy. The effect of various seed pre-treatments (tap water, gibberellic acid 500 ppm, stratification, sulphuric acid 98%, boiling water and nicking) were examined against the untreated (control) in the laboratory of Mizoram University Forestry Department. Both physical (exogenous) and physiological (endogenous) inhibition are likely to be the cause of dormancy in the seeds of *P. timoriana.* Our results showed more positive skewed towards physiological dormancy, as tap water, GA_3 and cold treatment (stratification) gave better result. However, acid scarification, boiling water and nicking little affect the germination and initial growth parameters. Our study recommends the use of simple tap water in *P. timoriana* seeds for enhancing seed germination and better yield of seedlings.

The third objective of the study was to see the effect of seed source and seed size/mass on seed germination and seedling growth. The effect of seed source on germination and seedling growth was already discussed along with variance component analysis (first objective) and therefore it will not be reviewed here. Seeds of *Parkia tiamoriana* show both intraspecific and interspecific variation in seed weight (Hopkins, 1986). A research was carried out to study the effect of seed mass on germination and early growth parameters of the species. Mature seeds were collected from Sakawrtuichhun provenance of Mizoram (India). They were then bulked and grouped into 3 categories as light (lwt), intermediate (mwt) and heavy (hwt), using a predetermines weight method. The grouped seeds are then sown using 1mm sieved garden soil as a medium in poly bags. After germination and from the two leave stage we start counting the seedling length, collar diameter, dry weight, etc., at every 15 days interval and up to 90th day, by using destructive method. Study on the germination and seedling growth parameters conclude that except in mean germination time (MGT) and germination index (GI), all the other parameters are positively correlated with increasing weight. Relative growth rate (RGR) and average growth rate (AGR) that use seedling dry weight also showed a positive relation with seed weight. Apart from this, the distribution pattern of seed weights as calculated from the frequency distribution of 255 seeds did not show lognormal distribution (K-S test: $P<0.05$, $d = 0.163$, $n = 255$). Seed weight ($n=255$) varied from 0.39g to 0.81g (mean: 0.61g ± 0.01g). Among the weight class, mid-weight (0.5 to 0.69g) seeds made up 56.47% of the total population followed by heavyweight (23.14%) and then by lightweight (20.39%).

The fourth objective was on seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* in Northeast India. Provenances distribution of this species was identified and clustered into different agro-climatic zones following Thornthwaite climatic model. The model is based on moisture index (MI) which is derived from potential evapotranspiration (PEP) and precipitation of the sites. The resultant agroclimatic zones were analysed for any significant difference or relation by taking all the important quantitative traits of seed and pod including their contribution to germination and growth. Analysis of variance showed significant variation ($p<0.05$) in all seed and pod traits of *P. timoriana* between agroclimatic zones. Polynomial regression drawn for the pod and seed characters against agro-climatic zones showed that there is gradual increase in pod length, pod weight, seed weight per pod, seed number per pod and 1000 seed weight, as aridity level increases from perhumid to arid. Interestingly, zonal performance in terms of germination and vigour also followed a similar trend with that of the weights of seed and pod; Arid > Humid > Subhumid > Perhumid. Therefore, we could conclude that tree breeders should choose seeds of *P. timoriana* from arid zone (MI = -20 to -60) in establishing a future seed orchard for distribution to villagers of other agroclimatic zones. Further, the zonal distribution with its corresponding moisture index was then interpolated to generate the respective agro-climatic map by using ARCGIS interpolation tool. This will help future researchers to identify and predict the better seed source easily before any plantation programmes.

Finally, the fifth and last objective was on Developing stem volume equation for *Parkia timoriana* (DC.) Merr. grown in home gardens and shifting cultivation areas of North-East India. This study aimed to establish a best fit volume model to facilitate estimation of biomass and carbon stock of the species without destructive sampling. Stem volumes of 360 trees representing 12 populations were estimated and the resultant data were used to develop nineteen commonly used volume models involving diameter at breast height (D) and height

(H) related variables. These developed models were then subjected to statistical test and then cross-validated to select the best fitting model. Coefficient of determination (R^2), root mean square error (RMSE), mean absolute deviation (MAD) and Akaike's Information Criterion (AIC) were used for model selection and scattered plot with residuals along with paired t-test were used for validation of the resultant models. Considering the validity and fitting statistics $V = 0.125 - 2{,}40265D + 21.43248D^2$ was found to be the best model using a single independent variable (D) while $V = 0.03177 + 1.032D^2H$ was the best model considering two independent variables (D and H). These two models gave high accuracy and hence can be used for future reference.

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PHOTO PLATES

Plate 1. Flower and pod development

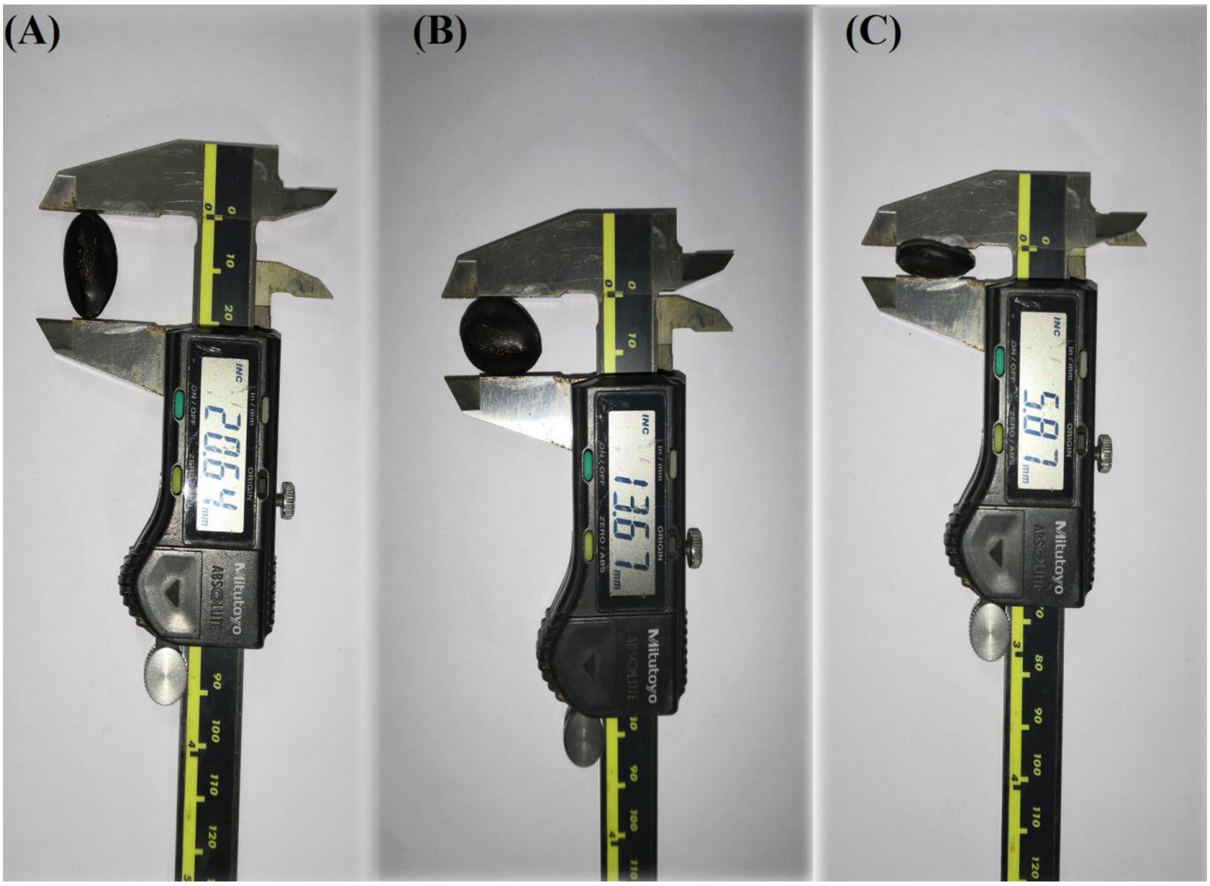

Plate 2. Seed characters: (A) Seed length, (B) Seed width and (C) Seed thickness

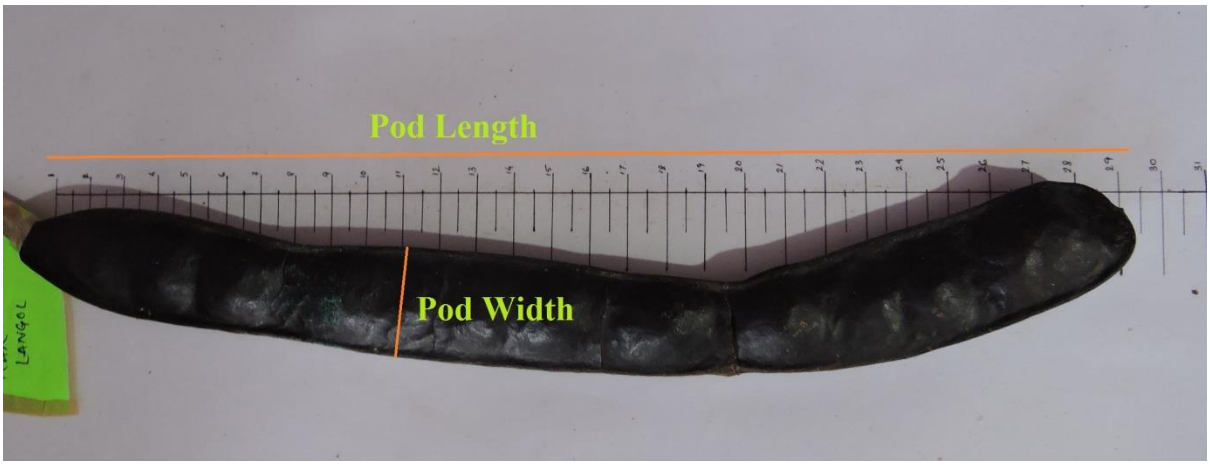

Plate 3. Pod characters

Plate 4. Seed germination assessment

Plate 5. Seedling Growth assessment

Plate 6. Field work for volume measurement

Plate 7. Soil analysis

BIODATA

Name:	Uttam Thangjam
Father's Name:	Thangjam Bhogendrajit Singh
Mother's Name:	Thangjam Sorojini Devi
Date of Birth:	11th March 1987
Nationality:	Indian
Permanent Address:	Sagolband Sayang Pukhri Mapal Imphal West, Manipur. 795001

Educational Qualification:

Year	**Name of Degree/Qualification**	**University/ Board**	**Subjects**
2002	Matriculation	Manipur Board	All
2004	Higher Secondary	Manipur Board	PCBM
2008	Graduation	H.N.B. Garhwal University, Srinagar	Forestry
2010	Post-Graduation	Forest Research Institute University, Dehradun.	Forestry (Wood Science and Technology)
2013	National Eligibility Test (NET)	Agricultural Scientist Recruitment Board (ASRB)	Forestry (Agroforestry)
2013-2019	Ph.D.	Mizoram University	Forestry

Ph. D. Topic: Provenance trial of *Parkia timoriana* (DC.) Merr. in North-east India.

List of Publication(s)

Sr. No	Title with Page nos.	Journal	ISSN/ ISBN No.	First/ co-author
1	Effect of Seed Mass on Germination and Seedling Vigour of ***Parkia timoriana*** (D.C) Merr.4(2):171-178, 2016	Current Agriculture Research Journal	ISSN-2347-4688	First author
2	Effects of different pre-treatments and germination media on seed germination and seedling growth of ***Parkia timoriana*** (D.C) Merr. 5(1):98-105, 2017	Journal of Experimental Biology and Agriculture Science	ISSN-2320-8694	First author
3	Floristic Composition And Regeneration Status Of ***Emblica Officinalis*** Gatern. In Two Semi-Evergreen Forest Stands Of Manipur, India. 6(3):563-571,2018	Journal of Experimental Biology and Agriculture Science	ISSN-2320-8694	Co-author
4	Endophytic fungi associated with ***Parkia timoriana*** (DC.) Merr – an edible legume tree of Northeast India. 9(1):57-63, 2018	NeBIO	ISSN 2278-2281	Co-author
5	Spatial and temporal dynamics of shifting cultivation in Manipur, NortheastIndia based on time-series satellite data. 14: 126-137,2019	Remote Sensing Applications: Society and Environment	ISSN 2352-9385	Co-author
6	Effect of agroclimate on seed and seedling traits ofTree bean (*Parkia timoriana* (DC.) Merr.) in North East India. 6(1):17-26, 2019	Indonesian Journal of Forestry Research	ISSN 2355-7079	First
7	Developing tree volume equation for *Parkia timoriana* grown in home gardens and shiftingcultivation areas of North-East India. DOI:	Forests, Trees and Livelihoods	ISSN 1472-8028	First

	10.1080/14728028.2019.1624200			
8	Changing trends of Shifting cultivation and its drivers in Champhai, Northeast India. 32(1):1-4,2019	Indian Journal of Hill Farming	ISSN: 0970-6429	Co-author
9	Influence Of Socio-Economic Factors On The Existing Shifting Cultivation Practice In Champhai District Of Mizoram, India. 9(3):325-330,2018	Journal of Hill Agriculture	ISSN: 0976-7606	Co-author

Paper(s) communicated

Sr. No	Title with Page nos.	Journal	ISSN/ ISBN No.	First/ co-author
1	Effect of provenance on morphometric, reproductive and seedling traits of ***Parkia timoriana*** (D.C.) Merr in North east India	Silva Fennica	ISSN: 2242-4075	First author

Paper(s) presented in Workshop/Seminar

Sr. No	Title of the Paper	Title of the workshop/seminar	Organized by	National or International
1	Seed Germination and initial seedling traits of ***Parkia timoriana*** (DC.) Merr. as affected by different pre treatments	International Conference on Natural Resources Management and Technology Trends	Centre of Advanced Study, Dept. of Life Sc., Manipur University, Imphal & SLNA Planning Dept. Manipur	International
2	Effect of seed source on reproductive traits of ***Parkia timoriana***	International Conference on Natural Resources Management for	Department of geography and resource management, School of earth	International

	(DC.) Merr.	sustainable development and rural livelihoods	science and NRM, Mizoram University, Mizoram	

Seminars/ Symposia/ Course/ Workshop Attended

S. No.	Title of symposium/orientation/workshop/ short term course attained	Place	Year	Duration (Days)
1	Interaction programme for Ph.D. scholars	MZU, Aizawl	2013	19
2	Workshop on applied statistics	MZU, Aizawl	2013	6
3	Additional course on disaster management	MZU, Aizawl	2014	21
4	Workshop on “Statistical and Computing Methods for Life-Science Data Analysis”	PUC, Aizawl	2015	8
5	Basic course on “Remote Sensing, GIS and GNSS”	MZU, Aizawl	2015	110
6	Pre-symposium tutorial on Watershed Management in Mountainous Landscape	IIRS, Dehradun	2016	2
7	National symposium on “Recent advances in Remote Sensing and GIS with Special Emphasis on Mountain Ecosystem”	IIRS, Dehradun	2016	3
8	Basic course on “Remote Sensing and GIS Application in Carbon Forestry”	MZU, Aizawl	2017	23
9	International Conference on Natural Resources Management and Technology Trends	MU, Imphal	2017	3
10	International conference on Natural Resources Management for Sustainable Development and Rural Livelihoods	MZU, Aizawl	2017	3

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE	:	Uttam Thangjam
DEGREE	:	Ph.D
DEPARTMENT	:	Forestry
TITLE OF THESIS	:	Provenance trial of *Parkia timoriana* (DC.) Merr. in North-East India
DATE OF ADMISSION	:	07.08.2013
APPROVAL OF RESEARCH PROPOSAL	:	02.05.2014
BOS	:	17.04.2014
SCHOOL BOARD	:	02.05.2014
REGISTRATION NO.& DATE	:	MZU/Ph.D./656 of 02.05.2014
EXTENSION (IF ANY)	:	Not taken

Head
Department of Forestry

PROVENANCE TRIAL OF *PARKIA TIMORIANA* (DC.) MERR. IN NORTH-EAST INDIA

THESIS SUBMITTED TO MIZORAM UNIVERSITY IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN FORESTRY

By

UTTAM THANGJAM

(Ph.D. Regn. No. MZU/Ph.D/656 of 02.05.2014)

ABSTRACT

DEPARTMENT OF FORESTRY

MIZORAM UNIVERSITY

AIZAWL-796004

ABSTRACT

Provenance represents the original native place where the seeds are sourced. Provenance trial focuses on selecting the best performing provenance by comparing its characters, or it can also be used for selecting the best characters by comparing the provenance. Both of which could be used in tree improvement programme. Effect of seed source variation and provenance trials are a common area of research for herbs and shrubs but are rare for trees.

P. timoriana is an important agroforestry tree species which is widely grown in home gardens and shifting agriculture lands of North-east India. The plant provides a good source of livelihood for both hill and valley people during winter and early spring season. Pods and seeds are taken as food and are sold at high price fetching a market value ranging from Rs. 70 to 120 Kg^{-1} (Firake et al., 2013). Other important uses of the tree include antioxidant property, ethnomedicinal and cosmetic uses, and firewood supply for the energy-deficient hill people (Angami et al., 2017). Along with its wide uses, there is an urgent need of research on adaptive potential and regeneration ecology as report on dying of this tree is increasing year after year. Insect pests such as *Cadra cautella Walker* (Thangjam, 2016), fungus and tree borers damage heartwood, pod and seeds causing dieback of the tree.

Adaptive potential of every tree is responsible for the change in character which in turn is supplemented by difference in genotypes, environments and their interactions (Savolainen et al., 2011). In forest and plantations of Asia-pacific region, *P. timoriana* has shown variations in its morphometric characters. This variation maybe because of the result of evolutionary history characteristics, mating system, population density and gene flow mechanism (Hamrick, 2004). *P. timoriana* like many other species of Mimosoideae have self-incompatibility. Pollen of this flower could not successfully fertilize with the ovule of the

ovary directly without any external help. Hence, large gene flow is expected viz-a-viz genetic variation among the population. Moreover, natural habitat range of *P. timoriana* varies extensively from foothill to elevation upto 1300 m above sea level within northeast India (Hopkins, 1994) and thus, one may expect genetic divergence among its populations in several traits. Though there are reports on variations in some of the traits of *P. timoriana* in different regions, there is still a lack of deeper scientific research on the individual traits representing individual characters for heredity and regeneration. Moreover, no studies have been made so far on the relation between provenance, maternal environment and germinability and seedling growth of the species.

Germinability and seedling growth are also affected by the type of dormancy and variation in seed weight. Normally, many trees exhibit seed dormancy so as to have a better chance of survival during unfavourable conditions. However, for plantation programmes, artificial favourable conditions are given to the seeds to overcome dormancy. Like many other leguminous trees, *P. timoriana* have hard-coated seeds. Under natural conditions, the seeds may take a much longer period to germinate thus necessitating use of pre-treatments (Aref et al., 2011) to increase the percent germination, rate of seed germination and finally seedling vigour. Seeds of this species also showed variations in mass and size. Seed mass represents a complex adaptive compromise (Harper, 1977). It plays a vital role in the establishment of the juvenile phase of a plant's growth curve. Different species give different results on the effect of seed mass on germination. Maximum work on this topic resulted in the more positive effect of larger seed mass, which might be because of the larger food reserve present. However, negative relationship between seed mass and relative growth rate were also reported in some species (Castro et al., 2008).

Another area of study of provenance is the effect of seed zones on germination and growth. Since *P. timoriana* has wide geographical distribution their provenance may represent a distinct agroclimatic zone. Many workers have tried to find the relationship between seed source or seed zones on germination and growth both within species and between species on many species (Aigbe et al., 2016; Moya et al., 2017), however, very little quantitative estimates regarding the role of species' adaptation on different agro-climates is done on this genus and none on this species. An experiment is designed using climatic model of Thornthwaith (1948) climatic classification and further mapping with ArcGIS interpolation tool, which fit the provenance related climatic data of *P. timoriana* under various agroclimatic zones.

P. timoriana, being dominant species in home gardens and shifting cultivation lands in North-east India, it is assumed that the species is a potential carbon sink for this region. This research also tried to generate the best fit volume model of *Parkia timoriana* by adopting non destructive process with diameter at breast height and height as independent variables, which could further be used to estimate biomass, carbon stock and carbon sequestration in the near future. Determining biomass and carbon stock is important as this will give an estimate of the amount of carbon that has been sequestered or emit when per unit of the tree biomass is formed or destroyed.

Assuming that variation in morphometric, germinative and seedling traits are influenced by provenance and environmental variation, we made an attempt to relate the available morphometric trait variations of this species with the geographic variations and seed source, and to identify best seed source to counter the dying population of this species which could help to develop a cultivar having incorporated all its desired characters. Thus, the present work was carried out with the following objectives:

1) Effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana* (DC.) Merr.
2) Effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana* (DC.) Merr.
3) Effect of seed source and seed size/mass on seed germination and seedling growth
4) Seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* in North-east India
5) Developing stem volume equation for *Parkia timoriana* (DC.) Merr.

The first objective of the thesis was about the effect of provenance on growth behaviour, phenology, pod and seed characteristics of *Parkia timoriana*. For this, 12 provenances representing 4 north-eastern states of India (Nagaland, Meghalaya, Manipur and Mizoram) were identified and selected for the present study for analysing the variation in different quantitative traits. The identified provenances were: (P1) Pherema, (P2) Medziphema, (P3) Shillong, (P4) Sumer, (P5) Bishnupur, (P6) Senapati, (P7) Jiribam, (P8) Langol, (P9) Achanbigei, (P10) Serchhip, (P11) Lunglei, and (P12) Sakawrtuichhun. Only mature trees of 12±2 years were taken for this experiment to maintain homogeneity in the result. Correlation coefficients were calculated for all possible geo-climatic traits, seed and pod related traits (SPT's), germinability and seedling growth traits. To draw an analogy between the magnitude of variation caused by genotype and environment, genetic/provenance coefficient of variance (PCV) and environmental coefficient of variance (ECV) were estimated. Broad sense heritability (h^2) was also calculated to determine the percent of phenotypic variation contributing to the total variation. It was observed that morphometric, germinative and seedling parameters vary considerably between provenances of *P. timoriana*. The overall ranking of the performances found P1 as the best among seed traits, P7 as the best for pod traits, P12 as the best for germination percent and P10 as the best for seedling vigour. On the other hand effect of provenance on morphometric, germinative and seedling growth indicates the influence of geo-climate and other genetic factors on it. Estimates of genetic and environmental effects indicate that genetic coefficient of variance (PCV) was higher for germination percentage (GP), germination energy (GE) and collar diameter (CD). In all these characters environmental factors were found to play minimal role as 75 to 95 % of the total variation was because of provenance variation as shown by the value of broad-sense heritability. Pod characters have a significant negative correlation with winter rainfall. While characters such as SWP and SWT were found increase with summer temperature. Heavier pods are also associated with lower latitude areas. Seeds from lower altitude tend to give larger seedling collar diameter.

The second objective of the study was to determine the effect of different pre-treatments on seed germination and seedling vigour of *Parkia timoriana*. Like many other legumes, *P.timoriana* also have hard-coated seeds which sought the need to investigate the most appropriate way to break its dormancy. The effect of various seed pretreatments (tap water, gibberellic acid 500 ppm, stratification, sulphuric acid 98%, boiling water and nicking) were examined against the untreated (control) in the laboratory of Mizoram University Forestry Department. Both physical (exogenous) and physiological (endogenous) inhibition are likely to be the cause of dormancy in the seeds of *P. timoriana.* Our results showed more positive skewed towards physiological dormancy, as tap water, GA_3 and cold treatment (stratification) gave a better result. However, acid scarification, boiling water and nicking little affect the germination and initial growth parameters. Our study recommends the use of simple tap water in *P. timoriana* seeds for enhancing seed germination and better yield of seedlings.

The third objective of the study was to see the effect of seed source and seed size/mass on seed germination and seedling growth. The effect of seed source on germination and seedling growth was already discussed along with variance component analysis (first objective) and therefore it will not be reviewed here. Seeds of *Parkia timoriana* show both intraspecific and interspecific variation in seed weight (Hopkins, 1986). A research was carried out to study the effect of seed mass on germination and early growth parameters of the species. Mature seeds were collected from Sakawrtuichhun provenance of Mizoram (India). They were then bulked and grouped into 3 categories as light (lwt), intermediate (mwt) and heavy (hwt), using a predetermines weight method. The grouped seeds are then sown using 1mm sieved garden soil as a medium in poly bags. After germination and from the two leave stage we start counting the seedling length, collar diameter, dry weight, etc., at every 15 days interval and up to 90th day, by using destructive method. Study on the germination and seedling growth parameters conclude that except in mean germination time (MGT) and germination index (GI), all the other parameters are positively correlated with increasing weight. Relative growth rate (RGR) and average growth rate (AGR) that use seedling dry weight also showed a positive relation with seed weight. Apart from this, the distribution pattern of seed weights as calculated from the frequency distribution of 255 seeds did not show lognormal distribution (K-S test: $P<0.05$, $d = 0.163$, $n = 255$). Seed weight ($n=255$) varied from 0.39g to 0.81g (mean: 0.61g ± 0.01g). Among the weight class, mid weight (0.5 to 0.69g) seeds made up 56.47% of the total population followed by heavy weight (23.14%) and then by light weight (20.39%).

The fourth objective was on seed source characteristics using Thornthwaite model and its effect on seed and seedling traits of *Parkia timoriana* in North-east India. Provenances distribution of this species was identified and clustered into different agro-climatic zones following Thornthwaite climatic model. The model is based on moisture index (MI) which is derived from potential evapotranspiration (PET) and precipitation of the sites. The resultant agroclimatic zones were analysed for any significant difference or relation by taking all the important quantitative traits of seed and pod including their contribution to germination and growth. Analysis of variance showed significant variation ($p<0.05$) in all seed and pod traits of *P. timoriana* between agroclimatic zones. Polynomial regression drawn for the pod and seed characters against agro-climatic zones showed that there is a gradual increase in pod length, pod weight, seed weight per pod, seed number per pod and 1000 seed weight, as aridity level increases from perhumid to arid. Interestingly, zonal performance in terms of germination and vigour also followed a similar trend with that of the weights of seed and pod; Arid > Humid > Subhumid > Perhumid. Therefore, we could conclude that tree breeders should choose seeds of *P. timoriana* from arid zone (MI = -20 to -60) in establishing a future seed orchard for distribution to villagers of other agroclimatic zones. Further, the zonal distribution with its corresponding moisture index was then interpolated to generate the respective agro-climatic map by using ARCGIS interpolation tool. This will help future researchers to identify and predict the better seed source easily before any plantation programmes.

Finally, the fifth and last objective was on Developing stem volume equation for *Parkia timoriana* (DC.) Merr. grown in home gardens and shifting cultivation areas of North-East India. This study aimed to establish a best fit volume model to facilitate estimation of biomass and carbon stock of the species without destructive sampling. Stem volumes of 360 trees representing 12 populations were estimated and the resultant data were used to develop nineteen commonly used volume models involving diameter at breast height (D) and height (H) related variables. These developed models were then subjected to statistical test and then cross-validated to select the best fitting model. Coefficient of determination (R^2), root mean square error (RMSE), mean absolute deviation (MAD) and Akaike's Information Criterion

(AIC) were used for model selection and scattered plot with residuals along with paired t-test were used for validation of the resultant models. Considering the validity and fitting statistics $V = 0.125 - 2,40265D + 21.43248D^2$ was found to be the best model using single independent variable (D) while $V = 0.03177 + 1.032D^2H$ was the best model considering two independent variables (D and H). These two models gave high accuracy and hence can be used for future reference.

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