A seed is an end and a beginning and bearer of the essentials of inheritance. It represents multiplication and dispersal, continuation and innovation, survival, renewal and birth. Seed is the most important propagule for regeneration of the sexually reproducing plants. The seed containing the embryo as the new plant in miniature is structurally and physiologically equipped for its role as a dispersal unit. It is also well provided with food reserves to sustain the growing seedling until it establishes itself as a self-sufficient, autotrophic organism. In nature, seeds pass from the plant through the very temporary environment of the distributing agency to a place on or in the ground where they will probably lie dormant for a while and where they will germinate under the favourable conditions and grow into new plants.

Species differ in their reproductive traits such as the minimum size at which they start to reproduce (van Ulf 2004), frequency of reproduction (Janzen 1978), quantity and size of seed produced (van Ulf 2004) and the dispersal mechanism and dispersal distance (Willson 1993). Various environmental factors that differ among habitats such as temperature, humidity, light, soil characteristics, dispersal syndromes, germination time, densities of competing plants, herbivore and fungi affect the production and selection for different seed sizes (Khurana & Singh 2001). Seed predation by insects, birds, mammals and damage by pathogens are important factors that affect regeneration of a particular species. Pathogens are an important cause of mortality for tropical seedlings and their effect is aggravated in moist, heavily-shaded habitats (Augsburger & Kelly 1984a). On the other hand, attraction of fruits and seeds by
mammals, birds and insects help in dispersal of different kinds of fruits at different degrees (Roosmalen 1985, Brewer & Rejmanek 1999). According to some theories, the dispersal of fruits or seeds away from the parent tree can enhance their chance of arriving and surviving in a suitable microenvironment and escaping mortality near the parent (Janzen 1970, Howe & Smallwood 1982, Hughes et al. 1994).

Viability and germination are two important events in the life cycle of a plant. On the basis of viability, seeds are classified into two categories: a) orthodox seeds those are usually dormant and can retain their viability for longer periods even under fully hydrated oxygenic condition and b) recalcitrant seeds those are having little or no dormancy and can not be dried below a critical moisture content (Roberts 1973). Seeds may have evolved thick seed coat as defensive structures for preventing penetration of insect ovipositors and the phenolic compounds in the seed coat contribute to the inhibition of microorganisms (Janzen 1969, Mohamed-Yasseen et al. 1994). Such seed coat induced dormancy can be broken artificially by mechanical scarification, hot water, dry heat or acid or other chemical treatments. Successful germination involves complex interactions between the seed’s physical position, its physiological state and its microenvironment (Hamrick & Lee 1987).

Information on the ecological requirements at the seed and seedling stages is vital both for conservation and rehabilitation and is seldom articulated in silvicultural and forest management plans (Guariguata 2000). Knowledge on ecology of germination and seedling growth is critical, not only for understanding the community processes of plant recruitment and succession, but also for developing strategies for the conservation of biodiversity and restoration of tropical forests (Khurana & Singh 2001).
Materials and Methods

Pod and seed collection: Pods were collected from 7 trees of three sites (Dambla village, Moishing and Yewang village) during January-February when they were mature and ready for shading. These were then carried to the laboratory for different experiments. Composite collection of 100 fruits from three sites was opened to count the number of seeds per fruit and construct a fruit size distribution curve. This was compared with log-normal distribution and tested for probability with Kolmogorov-Smirnov test (STATISTICA version 6.0).

Seed dimensions and weight: Pooled samples of seeds from all the 7 trees were used for measurements of seed dimension and fresh weight. Dimension of seeds were measured with the help of digital caliper (Mitutoyo, Japan). Total of 257 freshly collected seeds were weighed individually in electronic balance to generate a seed mass distribution curve and was compared with a normal distribution and tested by the Kolmogorov-Smirnov test (STATISTICA version 6.0) (d=0.093078, P=0.01).

Seed morphology and anatomy: Seed morphology was examined through visual and microscopic observation. External as well as internal seed structures were also observed under stereo microscope.

Scanning electron microscopy (SEM): Surface features of dry seed were examined with scanning electron microscope (SEM). For this, small section of seed coat was excised, mounted on metal stubs and gold-coated. Images were taken under different magnifications with scanning electron microscope (LEO 1430VP, Karlzeiz, German).

Seed viability, germination and moisture content: Seed dormancy and loss of viability was tested at 3 months interval for one year. For this, seeds were
stored in paper bags under laboratory conditions (darkness, temperature 20±5°C) until they were used. Seed viability was assessed with the tetrazolium test (MacKay 1972). In this test, formation of red coloured formazan in the embryo tissue is considered as an indicator of metabolic activity in the resting seed. Staining was done with 1% (W/V) freshly prepared aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (pH 7.0) at room temperature (25-27°C). Seeds were first imbibed in tap water, cotyledons were excised and placed in the test solution for 24 hours and scored as stained or unstained. Germinability was tested by placing seeds in Petri dishes over moist filter paper kept in normal condition. Germination was defined as the emergence of radicle from the seed coat. Newly germinated seeds were counted and removed from the dish. Observations were made for 60 days after which the overall germination rate was calculated. The tests were carried out at the campus of the North Eastern Regional Institute of Science and Technology (27°07’ N latitude, 93°22’ E longitude, 110 m asl).

Moisture content of seed was also estimated at 3 months interval by oven drying 50 seeds at 100°C for 48 hrs.

**Seed dormancy and different treatments to break dormancy:** Different treatments were given to the seeds to break seed-coat imposed dormancy. The treatments were 1) control (untreated seed) 2) mechanical scarification (the seed coat was sanded with wood sandpaper at the opposite of the embryo until the cotyledon was exposed) 3) hot water treatment (soaking of seeds in boiling water for 30 second and 1, 2, 3 and 5 minutes and 4) chemical scarification of intact seeds with concentrated sulfuric acid for 2, 5, 10, 20, 40, 60 and 120 minutes followed by washing in tap water for 10 minutes. Seeds from each treatment were germinated in Petri dishes with moist filter paper under normal laboratory condition (temperature 20±5°C and 12-hour photoperiod).
**Seed germination at different soil depth:** Seeds were germinated in polythene bags filled with farmyard soil (pH 5.4, Nitrogen 0.3%, Organic matter 4.3%) kept in mist chamber. Seeds were sown at different soil depths (0 cm *i.e.* surface, 1cm and 2cm) to see the effect of soil depth on seed germination. Watering was done regularly to keep the soil moist throughout the experimental period. New germination was recorded everyday and continued for 60 days after which cumulative germination percentage was calculated.

**Seed dispersal and predation:** Seed dispersal, predation and damage by various agents were observed during peak fruiting season. Dispersal by natural means was also observed. Pathogen infected seeds from forest floor were brought to the laboratory and pure cultures were developed. Fungal pathogens were identified by staining the mycelia from pure cultured colonies using suitable stains and were observed under microscope. Identification was done by following manuals as well as other literature.

**Vegetative mode of regeneration:** Branches of equal length and diameter was cut and dipped in different concentrations of root initiating hormones (100, 200 and 500 ppm) such as IAA, IBA, NAA and 2,4-D to study the vegetative mode of regeneration. Treated cuttings were than put in polythene bags filled with farmyard soil and transferred to green house for rooting. The experiment was conducted during September – October at Dirang and was monitored regularly.

Statistical analysis was done following Zar (1974) and using software packages (MS Excel, SYSTAT, STATISTICA and ORIGIN).
Results

Pod and seed characteristics: The dimension of fruit and number of seeds per fruit of all the trees collected from 3 sites are almost similar. Fruits are 6-17 cm long having 23-35 x 18-23 mm dimension. Number of seeds per fruit ranged from 1-8 and most of the fruits (60%) are 5-7 seeded (Plate 7.1-A). Frequency distribution of seeds per fruit is almost normal (Figure 7.1).

Seeds are 17.24 mm by 15.21 mm in dimension and are very hard. Seed weight varied from 1.2-2.4 gm and majority of the seeds were having weight range between 1.6-2.2 gm. Seed weight distribution was found negatively skewed having majority of seeds in heavier weight class (Figure 7.2).

Seeds are ovoid in shape and surface is fine textured (Plate 7.1-B). Outer black coloured testa is very hard and is impermeable in nature. However, crack on seed surface is well visible under microscope (Plate 7.1-C). There is a stony endodermal layer (when dry) inside the testa (Plate 7.1-D) which becomes mucilaginous after imbibition (Plate 7.1-E). Seeds are having two cotyledons with a basal embryo. There is a hollow cavity inside the cotyledons where the embryo lies (Plate 7.1-F).

Scanning electron microscopic observation showed very thick outer testa with marked cracks (Plate 7.1-G). Cracks were more in the vicinity of micropyle than the other areas. On the other hand, larger magnification of the same revealed densely cuticulated surface throughout the seed (Plate 7.1-H).

Seed viability, germination and moisture content: Seed showed very high percentage (90%) of viability immediately after harvesting. A considerably high percentage (46%) of seeds was viable even after 12 months of storage in normal laboratory condition (Figure 7.3). Germination percentage of fresh seed was also high (42%) which gradually decreased to 6.7% after 1 year of storage.

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Plate 7.1: A-Pod opened out; B-Mature seeds; C-Crack on seed surface; D-Seed-coat with hard testa; E-Mucilaginous content after seed imbibition; F- Seed in LS; G-SEM structure of seed surface; H- SEM structure of seed surface (higher magnification)
Figure 7.1: Frequency distribution of seeds per fruit (n=100). Seed number showed almost normal distribution.

Figure 7.2: Frequency distribution of seed weight of *G. assamicus*. The frequency distribution was negatively skewed.
Figure 7.3: Viability and germination response of *G. assamicus* seeds at different storage duration.
(Figure 7.3). On the other hand, loss of seed moisture content at 3 months interval for 1 year showed very little change (Figure 7.4)

**Different treatments to break dormancy:** Pretreatment of *G. assamicus* seeds by mechanical scarification, hot water treatment and chemical scarification by Sulphuric acid improved germination at different degrees. Whereas maximum improvement of germination (80%) was found in seeds treated with boiling water for 2 minutes against minimum germination in control experiment (42%). Other treatments such as mechanical and chemical scarification showed significant improvement in rapid and uniform germination as compared with the control (Figure 7.5).

**Seed germination at different soil depths:** Seed germination in *G. assamicus* was hypogeal as the cotyledons remain below ground after germination. Germination at different soil depths showed different results. Maximum percentage of seed germination (42%) was observed when seeds were sown at 1 cm soil depth while it was minimum (20%) at 2 cm depth (Figure 7.6). There was significant variation in seed germination percentage among different soil depths (F=13.038; P<0.05).

**Vegetative mode of regeneration:** Different hormone treatments for vegetative mode of propagation did not give any result. Though there was leaf initiation from the cuttings, no rooting was noticed and the branches died after a few days.

**Fungal pathogens identified:** Fruits of *G. assamicus* in forest floor were found largely infested by fungi especially by *Aspergillus* sp., *Mucor* sp. and *Humicola* sp. and contributed significantly in damage of mature fruits in soil.
Figure 7.4: Loss of seed moisture content during one year storage
Figure 7.5: Effect of various treatments [mechanical scarification, Salphuric acid (SA) and boiling water (BW)] on germination of *G. assamicus* seeds. 
1 = control, 2 = mechanical scarification, 3 = SA for 2 min, 4 = SA for 5 min, 5 = SA for 10 min, 6 = SA for 20 min, 7 = SA for 40 min, 8 = SA for 60 min, 9 = SA for 2 hr, 10 = BW for 30 sec, 11 = BW for 1 minute, 12 = BW for 2 minutes, 13 = BW for 3 minutes and 14 = BW for 5 minutes. (Vertical bar represents ± SE of the mean)

Figure 7.6: Seed germination percentage at different soil depth
Discussion

In the present observation, it was found that fruit dimension and number of seeds per fruit in all the sites is almost similar. This may be due to similar physiography and age structure of the individual trees in all the sites. On the other hand, it was observed that seed mass distribution curve is negatively skewed indicating more numbers of seeds with heavier class.

Seed surface morphology reveals that hard and impermeable testa is the main barrier for imbibition and consequently retards germination. Similar hard seed-coated dormancy is common in other legume also (Quinlivan 1971, Baskin & Baskin 1989, Thapliyal et al. 1998). Extreme hard seed coat in the mature dry state is generally due to the presence of heavily thickened galactomannan or mannan polymers on the walls of the endosperm cells and are well observed in Carob (Ceratonia siliqua L.) (McCleary & Matheson 1974) and Fenugreek (Trigonella foenum-graecum L.) (Reid 1971). The endosperm walls contain hydrophilic galactomannans which become mucilaginous after a period of imbibition and hydrolysis (Reid & Bewley 1979). Seeds of G. assamicus also become mucilaginous after imbibition and there are possibilities that galactomannan or mannan polymers are present in the endosperm wall. Hard seed coats with a palisade or malpighian layer in the Leguminosae and Malvaceae family is due to special devices which enable seeds to imbibe water preceding germination. Seed coats only become permeable after release or splitting of a special region of the malpighian layer (Graven et al. 1997).

Scanning electron microscopic observation showed very compact nature of testa which may prevent uniform intake of water throughout the seed surface. This may have led the seed highly impermeable in nature. More cracks on the vicinity of micropyle promote imbibition as well as rapid activation of the
embryo. SEM studies also revealed that seed coat of *G. assamicus* is having a continuous layer of tightly packed palisade cells. Hard-seededness due to tightly packed palisade cells in the seed coat might be the major barrier to water entry into seeds (Cavanagh 1987, Egley 1989). Though seed coat impermeability varies in most leguminous species, the lens (strophiole) forms a structural weakness which can be disrupted naturally or artificially, thus rendering the seeds permeable to water (Teketay 1997).

**Dormancy, seed viability and germination:** Seeds were viable for a long period of time (Figure 7.3) and such seed types are referred to as orthodox. Loss of seed moisture content at 3 months interval for 1 year showed very little change (Figure 7.4) indicating copious viability for longer duration. Such seeds with high initial viability and germination percentage retain their viability for longer than immature seeds (Stein *et al.* 1974). These observations suggest very easy and long-term preservation of mature seeds for successful seed germination and seedling raising for future implication.

**Breaking seed dormancy:** Very hard testa was found to be the main barrier for impermeability in case of *G. assamicus*. Seed coat impermeability is related to hard seed coat as well as the presence of waxy layer surrounding the cotyledons. The degree of seed coat thickening is influenced by genotype, conditions during the growing season and dehydration rate of the seed (Patane & Bradford 1993, Russi 1993). Results from the present study showed that seed coat imposed dormancy due to water impermeability in *G. assamicus* can be broken by mechanical scarification, boiling water or acid treatments (Figure 7.6). Similar results have also been demonstrated by several workers in different legume species (Larsen 1964, Khan & Tripathi 1987, Danthu *et al.* 1992, Masamba 1994). Interestingly, boiling water treatment improved germination significantly than other treatments. Similar result was also obtained in another legume *Acacia*
negrii (Teketay 1997). However, several other legume species responded poorly to boiling water treatments (Larsen 1964, Clements et al. 1977, Danthu et al. 1992). Implication for such relatively simple, convenient and cheap methods which requires little skill can also be practically applied for large scale seedling production and restoration measures.

Seed coat impermeability which is very common characteristics in most legumes is a delaying mechanism and prevents germination under unfavorable condition (Barton 1965, Tran & Cavanagh 1984, Baskin & Baskin 1989, Tybrik 1991). Such hard seed coat also helps to survive in the soil because seeds must not only remain viable, but also not to germinate before favourable period. Hard seedcoat imposed dormancy is common in perennial leguminous plants and may share up to 80% and more (Travlos et al. 2007). Orthodox seeds acquire desiccation tolerance during development and may be stored in the dry state for predictable periods under defined conditions. Hard seed coat also allows endozoic dispersal, recolonization after fire and helps the seeds to withstand unfavourable conditions such as heat, drought, digestive juice as well as mechanical damage (Tybrik 1991). Gymnocladus assamicus seeds were also found ingested by grazing animals and there are possibilities that passage through digestive tract improve germination and thus regeneration as well.

The pretreatment in the laboratory reflect the ways by which G. assamicus seeds overcome dormancy in nature. Permanent action of weak acidic soil in the study site softens the seed coat as presumed from laboratory treatments in strong acid. Chewing and ruminating the pods by animal may give mechanical scarification of seeds and improve imbibition as well as germination.

Various pretreatment methods of G. assamicus seeds showed significant improvement in rapid and uniform germination. However, boiling water
treatments for 2 minutes may be highly recommended for its low cost, convenience and ease in the use.

Germination experiments at different soil depths indicated that *G. assamicus* seeds germinate well at 1 cm depth rather than surface or 2 cm depth. Similar result was also obtained from other legume species (Bradstock & Auld 1995, Williams *et al.* 2004). It has been suggested that larger seeded species could benefit the most from increased temperature penetration in to the topsoil, because their larger starch reserves might allow survival of germinants from greater depths in the topsoil (Midgley & Bond 2001). *G. assamicus*, being a legume, is having seeds with rich nutrient content and might have developed a strategy for survival at greater depth.

Seeds remain viable for 1 year and the estimation revealed that 80% seed remain viable after 3 months (*Figure 7.3*) *i.e.* during June-July when the outdoor condition is the most favourable for seed germination. Seed germination with respect to soil depth showed that *G. assamicus* germinates best at 1 cm depth. Better germination for the New South Wales legume *Acacia suaveolens* was also recorded from medium soil depth (Bradstock & Auld 1995). This may be an advantage to escape predation and damage by pathogen as well as washout by rainwater.

**Dispersal:** As the seeds are of orthodox type, the species may be designated as fugitive in nature (Hutchinson 1951) and good dispersal mechanism would play an important role permitting the species to colonize new habitats (Salisbury 1942).

Seed predation by various mammals and birds are common in majority of the forest tree species. In the present study, major seed hoarding animals noticed were hill cattle, goats and arboREAL rodent. Fruit rots due to *Alternaria alternata* have been reported in citrus, apple and pear (Domsch *et al.* 1993) and in *Pinus*
*dendroflora* and *Cryptomeria japonica* seeds (Watanabe 2002). Seed rot by forest pathogens significantly inhibit natural regeneration of several species at seed stage (Cho *et al.* 2007). Fungi play an important role in seed survivorship in soil (Crist & Friese 1993, Leishman *et al.* 2000, Blaney & Kotanen 2001), and many fungi inhibit seed germination (Dinoor & Eshed 1984) and causes severe damage to the soil seed banks. In the present study, infection rate by *Aspergillus* and *Mucor* sp. was found highest in pods retrieved from the field.

As the size of a single species population is related to seed supply and largely determined by favourable conditions available for germination and establishment (Harper *et al.* 1979), the major constraints for extremely small population of *G. assamicus* may be due to poor seed supply, dispersal and poor germination.