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Seedling dynamics of the critically endangered tree legume *Gymnocladus assamicus* in northeast India

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Abstract: *Gymnocladus assamicus* is a critically endangered tree species in northeast region of India having very few or no regenerating populations. Conservation and management is an urgent need to protect the species from extinction. We studied seedling dynamics in *in-situ* and *ex-situ* conditions to develop suitable conservation strategies and artificial regeneration in different ecological conditions. For *in-situ* experiments, tagged seedlings were monitored periodically in natural conditions, while for *ex-situ* experiments, nursery grown seedlings were transplanted in different environmental conditions and studied for their survival and growth performances. The study revealed that *G. assamicus* seedlings were light-dependent and express optimum growth and survival under moderate exposure to sunlight. Sapling population was recorded exclusively beyond the canopy of the mother tree. However, lack of seed dispersal was identified as a major constraint for natural regeneration of the species. Seedling mortality was mainly due to water stress along with some degree of chilling affect of the cold winter in its native range. *Ex-situ* experiments demonstrated that survival and establishment of *G. assamicus* seedlings beyond its natural distribution range is extremely poor. Therefore, any initiative to re-introduce the species should be practiced in its home range with special emphasis to prevent water stress during dry season.

Resumen: *Gymnocladus assamicus* es una especie arbórea en peligro de extinción de la región noreste de la India que tiene muy pocas, o quizá ninguna, poblaciones que se estén regenerando. La conservación y el manejo son necesidades urgentesa fin de proteger a esta especie de la extinción. Estudiamos la dinámica de plántulas en condiciones *in situ* y *ex situ* para desarrollar estrategias adecuadas de conservación y regeneración artificial en diferentes condiciones ecológicas. Para los experimentos *in situ*, plántulas etiquetadas fueron monitoreadas periódicamente en condiciones naturales, mientras que para los experimentos *ex situ*, las plántulas que crecieron en vivero fueron trasportadas a diferentes condiciones ambientales, y se estudió su desempeño en términos de supervivencia y crecimiento. El estudio mostró que las plántulas de *G. assamicus* on dependientes de la luz y que expresansu crecimiento y supervivencia óptimoscuando la exposición a la luz solar es moderada. Las poblaciones de individuos jóvenes fueron registradas exclusivamente fuera de la copa del árbol madre. Sin embargo, la falta de dispersión de semillas fue identificada como un obstáculo importante para la regeneración natural de la especie. La mortalidad de plántulas se debió principalmente al estrés

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hídrico, y en cierta medidaalefecto de enfriamiento por el invierno frío en su área de distribución natural. Los experimentos *ex situ* demostraron que la supervivencia y el establecimiento de plántulas de *G. assamicus* fuera de su área de distribución natural son extremadamente bajos. Por lo tanto, cualquier iniciativa para reintroducir la especie se debe practicar en su área nativa de distribución, haciendo énfasis en tratar de evitarel estréshídrico durante la estación seca.

Resumo: A *Gymnocladus assamicus* é uma espécie criticamente ameaçada de extinção na região nordeste da Índia, tendo muito poucas ou nenhumas populações em regeneração. A conservação e gestão são uma necessidade urgente para proteger a espécie da extinção. Nós estudámos a dinâmica das plântulas em condiçõesin-situ e *ex-situ* para o desenvolvimento de estratégias de conservação adequadas e a regeneração artificial em diferentes condições ecológicas. Para os ensaios *in-situ*, as plântulas marcados foram monitoradas periodicamente em condições naturais, enquanto que para os ensaios *ex-situ*, as plântulas criadas em viveiro foram transplantadas para diferentes condições ambientais e estudadas quanto à sua sobrevivência e performances de crescimento. O estudo revelou que as plântulas de *G. assamicus* heliófilas e expressavam melhor o seu crescimento e sobrevivência sob exposição moderada à luz solar. A população juvenil foi registada exclusivamente fora da projeção da copa da árvore-mãe. No entanto, a falta de dispersão de sementes foi identificada como um dos principais entraves para a regeneração natural da espécie. A mortalidade de plântulas foi principalmente devida ao stresse hídrico, juntamente com algum efeito do frio de inverno na sua área nativa. Os ensaios *ex-situ* demonstraram que a sobrevivência, eo estabelecimento de plântulas de *G. assamicus*, para além de sua área de distribuição natural é extremamente pobre. Portanto, qualquer iniciativa de reintroduzir a espécie deve ser praticado na sua área natural de vegetação, com especial ênfase para evitar stresse hídrico durante a estação seca.

Key words: Conservation, growth, mortality, plantation, re-introduction, regeneration, survival.

Introduction

Gymnocladus assamicus Kanjilal ex P.C. Kanjilal (Leguminosae) is an endemic tree species in Northeast (NE) India. It was first described from the Khasi Hills of Meghalaya (earlier Assam) in 1934 (Kanjilal *et al.* 1938) and remained 'least documented' for several decades. The species was included in the priority list for species recovery program in India (Ganeshaiah 2005). Extensive field surveys were conducted in various parts of NE India including its type locality in Khasi Hills, however, only a few populations were reported from West Kameng and Twang districts of Arunachal Pradesh. We also employed ecological niche modeling and located 14 discrete populations of 1 to 7 trees each (Menon *et al.* 2010). Species of such limited distribution often faces acute regeneration failure leading to extinction. Therefore, studying regeneration status is important to understand the dynamics of a species in its natural condition.

Population dynamics of plant species can be described by demographic characteristics, such as the recruitment, mortality and growth rates of individuals (Watkinson 1997). Patterns of birth and death determine population size and vary drastically within species (Roff 1992). Balance among these variables has been found to regulate the dynamics and structure of plant population (Kohyama & Hara 1989). It was found that mature, hermaphrodite *G. assamicus* trees produce significant number of seeds (Choudhury 2008), however, population structure demonstrated that seedling and sapling populations do not contribute to the maintenance of population of the species. Seed-coat imposed dormancy was found to be a major constraint for natural regeneration (Choudhury *et al.* 2009).

Seedling phase is a very sensitive stage in a plant's life cycle. Therefore, successful regeneration largely depends upon the prevailing micro-environment (Cleavitt *et al.* 2011; Whitmore 1996). Photosynthetic active radiation (Jakovac *et al.*

2012), water availability (Whitmore 1990), pathogen (Mueller-Dombois *et al.* 1983), and herbivory (Latorre *et al.* 2013), among others, play major role in seedling growth and establishment. Members of the legume family show enormous diversity in their life forms and exhibit wide range of adaptations to cope up with the environment. These traits are expressed through remarkable levels of adaptive variation in morphological characters such as growth form, canopy architecture, root architecture as well as physiological features, for example, phenological controls, water relations, nitrogen fixation and mycorrhizal associations (Rundel 1989). Legumes adopted various dispersal modes (endozoochory, autochory etc.) and their almost ubiquitous presence of hard seed coats ensures maximum seed survival (when pods are consumed by animals) that contributes to the formation of permanent, long-lived soil seed banks (Arianoutsou & Thanos 1996). With all such advantages 'critically endangered' status of any legume taxa like *G. assamicus* is of serious concern. Therefore, adequate knowledge of seedling dynamics is essential to develop effective *in-situ* and *ex-situ* conservation strategies of such plant species. Re-introduction of plant species which have become endangered or extinct is an effective method used in conservation and management (Godefroid *et al.* 2011). However, no information about the seedling dynamics is available for *G. assamicus*, thereby limiting any successful re-introduction initiative for the species.

In the present study, we demonstrated various life history traits of the *G. assamicus* seedlings such as growth, survival and their responses to two different ecological conditions. Dirang and NERIST sites fall under Eastern Himalayan wet temperate forest (II-12B/CIa) and Assam alluvial plains semi-evergreen forest (2B/C1), respectively (Champion & Seth 1968). The study also attempted to address different morphological changes in the seedling phase, seedling survival and new recruitment, and growth performance in *in-situ* and *ex-situ* conditions.

Materials and methods

In-situ experiments

G. assamicus is a critically endangered legume tree species in NE India and was documented from only 14 populations of 1 to 7 trees each (Menon *et al.* 2010). Natural regeneration of the species was found very poor having seedling and sapling populations only at Moishing site (27°18′ N latitude; 92°14′ E longitude; 2052 m asl) in West Kameng District of Arunachal Pradesh (Fig. 1). Therefore, *in-situ* experiments were conducted only in Moishing site and no replicates could be performed. To understand the population dynamics in natural condition, seedling, sapling and tree population density were censused along the increase in distance from the mother tree trunk (Khan & Tripathi 1986). Seedlings from each of the 1 m radial increment (from mother tree trunks) were counted and numbered with permanently labeled aluminium tags in the month of April 2005. Survival of the seedlings were recorded every six months interval for two years. Atleast two to five seedlings per 1 m radial unit (depending upon the availability) were tagged, totaling 54 seedlings in the Moishing site.

Ex-situ experiments

The *ex-situ* experiments were conducted in two different locations to evaluate the response of the seedlings at different ecological conditions. The first site was at Dirang in West Kameng District of Arunachal Pradesh. The second site was at the Botanical Garden, Department of Forestry, North Eastern Regional Institute of Science and Technology (NERIST) located in Papum Pare District in Arunachal Pradesh (27° 07´ N latitude; 93° 22´ E longitude; 110 m asl). NERIST site was chosen because it has different climatic conditions than the native range of the species, has a large botanical garden for field experiment and easy accessibility for regular monitoring (as we were stationed at NERIST). Topography and climatic conditions between the two sites differ significantly (Table 1), (Fig. 2 A,B). The main objectives of the *ex-situ* experiments were to study the seedling performance in wider geographical regions and develop strategies for re-introduction of the species, for example, in roadside plantation or social forestry programs.

Phenological observations revealed that *G. assamicus* pods mature during January and persist in the tree until April (Choudhury 2008). Therefore, we harvested the mature pods at the end of January 2005 and brought to Dirang for further processing. Two hundred seeds from randomly chosen pods were removed and divided equally for Dirang and NERIST nursery. Our study revealed that more than 70 % of the freshly collected seeds were viable and were ready for germination (Choudhury *et al.* 2009). We sowed one

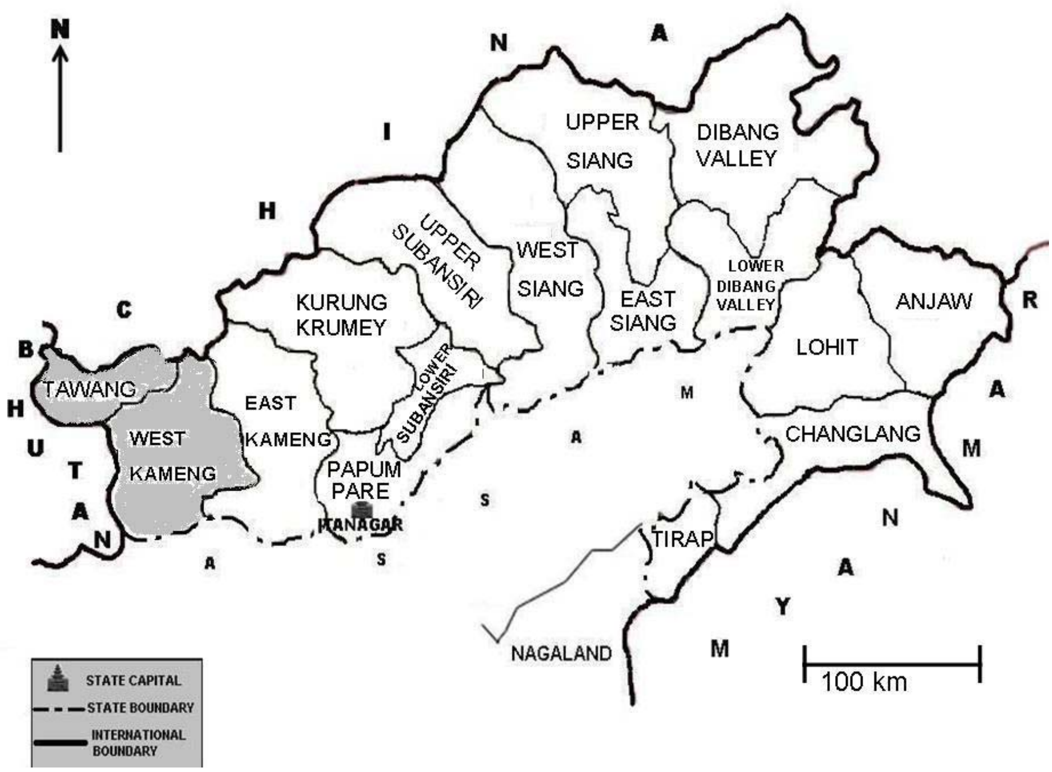

Fig. 1. Map Arunachal Pradesh showing the distribution of *G. assamicus* populations.

seed in each bag containing approximately one kilogram farmyard soil for germination and kept in greenhouse until the seedlings were ready for transplantation. Regular water supply was provided to minimize water stress during seedling growth.

Survival of the re-introduced seedlings in different habitats

About four months old nursery-grown seedlings were re-introduced at Dirang and NERIST Botanical Garden during April 2005 (onset of monsoon period). Local people at Dirang also assisted during transplantation in their home-gardens for its ethnobotanical uses. We labeled 50 seedlings with permanent aluminium tags and transplanted in each site. Growth and survival of the transplanted seedlings were monitored periodically over a period of two years. While transplanted seedlings at Dirang were visited at six months interval (because of remote location), NERIST seedlings were monitored and censused every month to record survival and growth performance. Photosynthetic active radiation (PAR) was measured with the help of LICOR plant canopy analyzer. Water holding capacity (WHC) was determined following the Keen's box method (Piper 1944) while soil moisture content was measured gravimetrically by incubating 10 g of field moist soil sample in a hot-air oven at 105 °C for 24 h. Rainfall and maximum-minimum temperature of the sites were collected from the State Meteorology Department and NERIST Meteorology Center during the study period. Regression analyses were conducted to understand the interaction between seedling mortality and different environmental factors such as rainfall, minimum and maximum temperature using the software program STATISTICA (StatSoft 1995).

Results

G. assamicus is a deciduous tree and remains completely leafless for over two months during dry winter (January-February). Fully mature pods persist on trees until next flowering season when the pods become ready to disperse by gravity. New leaves appear during early March followed by flowering during April and lasts only

Table 1. Site characteristics of different locations where seedling growth performances were studied (data recorded during 2005-2007).

Parameters	Natural	Nursery		Transplanted	
	Moishing	Dirang	NERIST	Dirang	NERIST
Mean altitude (m)	2052	1715	110	1715	110
Max/Min air temperature (°C)	1-34	1-34	20-38	1-34	20-38
PAR (µmol s^{-1} m^{-2})	38-45	500-540	130-180	1000-1100	1500-1700
Annual precipitation (mm)	1800-2000	1800-2000	1572-3076	1800-2000	1572-3076
Average soil moisture (%)	30-35	35-40	35-40	17-20	15-20
Water holding capacity (%)	60-65	60-65	60-70	45-50	40-45

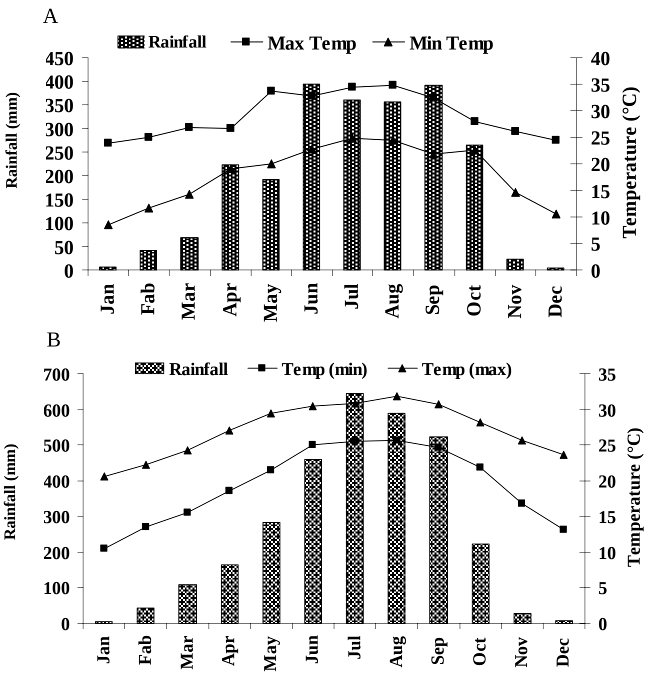

Fig. 2. Average monthly rainfall and temperature of (A) Dirang and (B) NERIST.

for 15-20 days. Seeds are ovoid, 17.24 mm by 15.21 mm in dimension and extremely hard. Seeds have a basal embryo and a tiny radicle inside the hollow cavity of the cotyledons. Other than the harvesting of mature pods by humans for its traditional use, damaged pods and seeds probably by squirrels or arboreal rats were found in the tree bog. Grazing animals often destroy mature pods by chewing and ruminating the seeds from forest floor. However, no seed dispersal agents could be observed during our study.

Seedling morphology

Depending upon the cotyledon exposure, seedling of *G. assamicus* has been described as phanerocotylar hypogeal with reserve cotyledons (PHR) type. On the other hand, depending upon the seedlings with storage reserves in the hypocotyl or root, seedling of *G. assamicus* may be classified as fleshy cryptocotylar type as the cotyledons are specialized haustorial organs which never emerge from the seed and has enough food reserves in the endosperm (Garwood 1996).

Demography of the seedlings in natural habitat

Seedlings of *G. assamicus* in Moishing site are distributed around the mother tree on a hill-slope along a small seasonal stream. First population census in 2005 revealed that Moishing site has 92.49 % seedling population followed by 6.36 % sapling and 1.16 % mature trees. Seedling and sapling populations were distributed up to 12 m radial distance from the mother tree trunk. Seedling population was more below the tree canopy having highest density (86 %) in 3 to 9 m radial distance (Fig. 3) while sapling population was observed only beyond the tree canopy. Survival and density of the saplings were recorded only in 8 to 12 m radial distance from the mother tree trunk. No seedling or sapling was recorded beyond 12 m radius suggesting very poor seed dispersal of *G. assamicus* in natural condition. Since only Moishing site was having seedling and sapling population, we could not perform further statistical analysis of the data for this study.

In-situ seedling dynamics

Two years of field study on survival and mortality revealed that around 36 % seedlings died each year and the mortality was higher during dry season compared to rainy season. This could be mainly due to dry and cold weather conditions throughout the winter. Around 23 % seedling mortality was recorded during the drier season (October to April) while mortality was

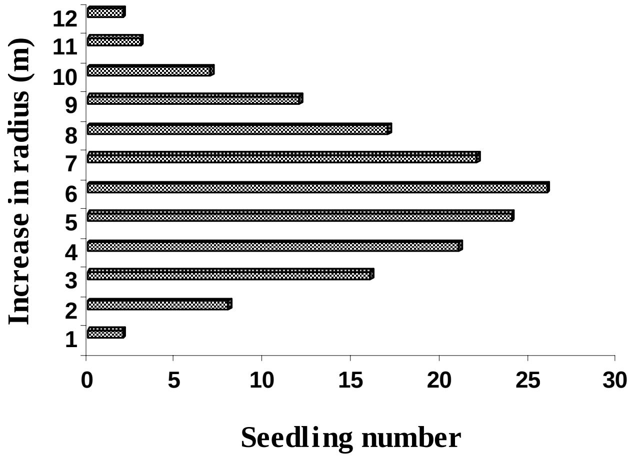

Fig. 3. Density of the *G. assamicus* seedlings along different radial distances from the parent tree.

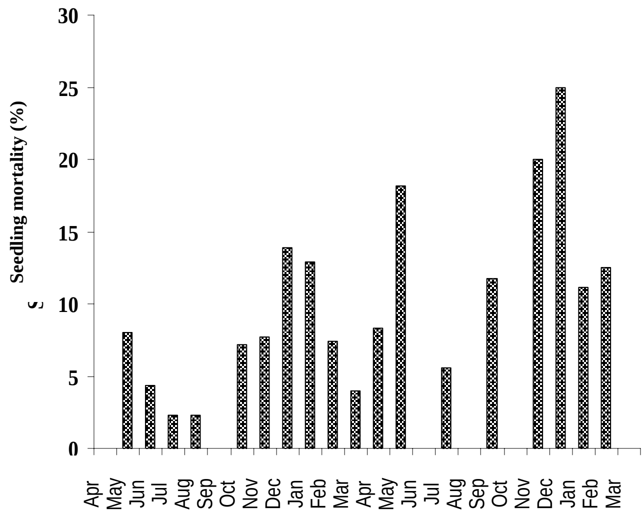

Fig. 4. Seedling mortality at NERIST Botanical Garden from April 2005 to March 2007.

around 12 % during rainy season of the year (May to September). Overall, 43 % of the original tagged seedlings died during the 2005-2006 study period. *G. assamicus* seedlings grew very slowly with an average of 0.53 cm (± 0.07) per year, which made it difficult to ascertain the approximate age of the seedlings. Seedling mortality was also studied along 1 m radial increment from the mother plant and found higher mortality below the tree canopy as predicted by the Janzen-Connell hypothesis (Connell 1971; Janzen 1970). Seedling survival at further distances from the mother plant is also evidenced from the occurrence of sapling population at 9 to 12 m radial distance.

Survival of transplanted seedlings

Survival of the transplanted seedlings in two sites showed remarkable difference in the present study. In the first year, 52 % seedlings mortality was recorded at the NERIST Botanical Garden while it was 46 % at Dirang site. Monthly census of the transplanted seedling population at NERIST site revealed that mortality is more during dry season and less during wet season (Fig. 4). Similar trend was also observed in Dirang site where 22 % and 18 % seedling mortality was recorded during 2005 and 2006 rainy seasons and 30.77 % mortality during the dry season. Multiple regression analysis showed that rainfall and minimum temperature had a significant ($P < 0.005$) correlation with the seedling mortality (Fig. 5 A,B). On the other hand, the correlation of maximum temperature with seedling mortality is not significant (Fig. 5 C).

Discussion

The present study on seedling ecology revealed a few crucial aspects particularly important in conservation and management of *G. assamicus* in *ex-situ* and *in-situ* conditions. It was found that seedlings of *G. assamicus* were PHR type based on their morphology. Among many others, the PHR seedling type has been found only among 7.2 % plant species, mostly belonging to the liana species (Ibarra-Manríquez *et al.* 2001). Lianas are light-dependent plants which grow and establish better in open forest areas or forest gaps (Hegarty & Caballé 1991; Putz 1984). This indicated that the *G. assamicus* seedlings are light dependant and their survival will depend largely on open canopy. Our observations further revealed that sapling populations were distributed only beyond the canopy of the mother tree, indicating more light requirement for their survival. Such behavior is also evidenced in many other forest tree seedlings (Castro-Marín *et al.* 2011; Marimon *et al.* 2012).

Demography of the seedling and sapling populations at Moishing revealed very poor seed dispersal mechanism in *G. assamicus*. Occurrence of significantly lower mature tree population than the seedling and sapling population could be mostly due to failure of survival of the regenerating individuals (Jacobs & Biggs 2002; Pant & Samant 2012). Though the seedling population was significantly ($P < 0.05$) higher at 4 m to 8 m radius under the parent tree canopy, sapling population was found only at 8 m to 12 m radial distance (Fig. 6). One of the reasons for this could be due to less competition beyond the parent tree (Connell 1971; Janzen 1970). Survival at greater

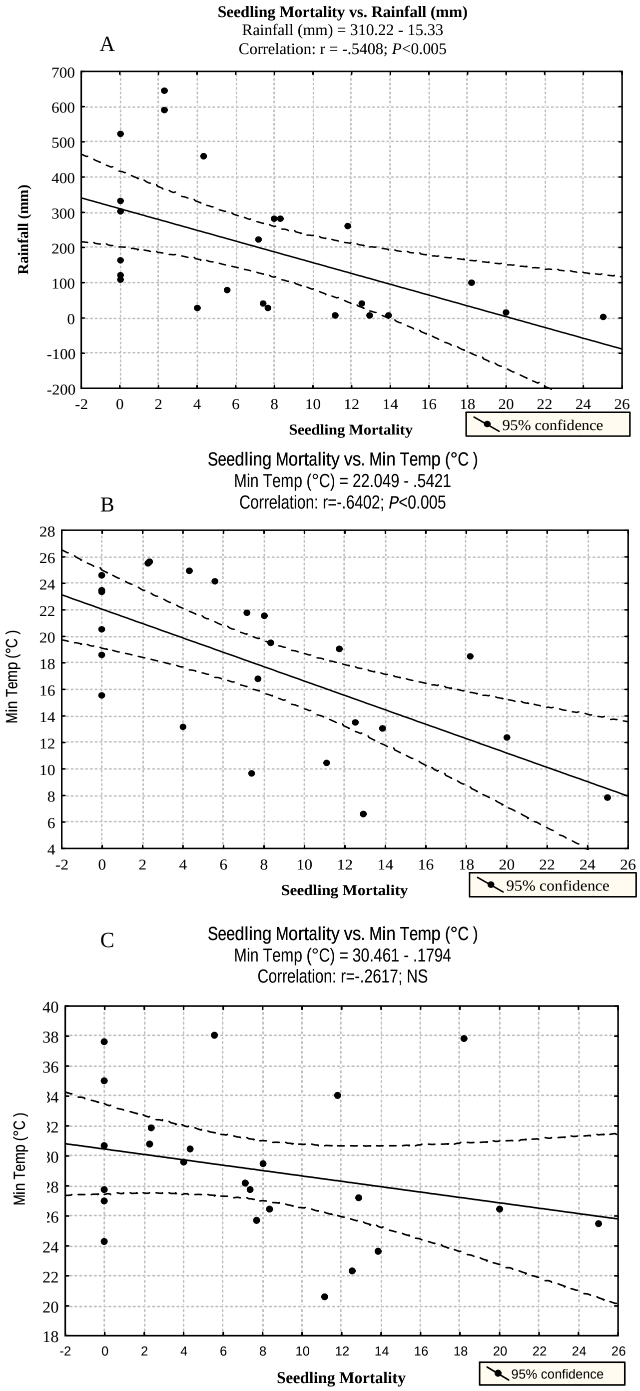

Fig. 5. Multiple regression analysis between seedling mortality and different environmental parameters in NERIST study site. (A) Regression between seedling mortality and rainfall; (B) Regression between seedling mortality and minimum temperature; and (C) Regression between seedling mortality and maximum temperature (NS = not significant).

distance may be due to lesser competition and spatial differences in micro-environment such as leaf litter and soil nutrients along the different distance. Because of very slow growth rate of *G. assamicus*, no seedling could have been found to attain sapling stage during the two years study period.

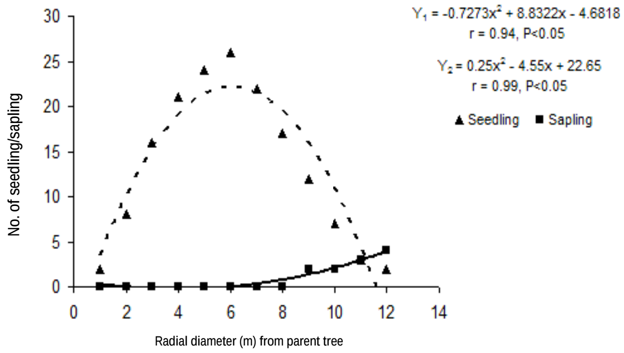

Fig. 6. Relationship between radial increase and seedling (Y_1)/sapling (Y_2) density.

Seedling growth performances in both the sites were superior during rainy season (April-September) compared to dry and cool season (October-March). Better seedling growth rates and survival during rainy season may be due to high soil moisture content and rapid decomposition of leaf-litter during that period (Devi 2004). Our study showed significant positive correlation between seedling mortality and decreased soil moisture as well as decrease in temperature during the dry and cold winter months. Soil moisture plays an important role in seedling growth and has been evidenced in many studies (Marimon *et al.* 2012; Mueller-Dombois *et al.* 1980; Quero *et al.* 2006). Seedlings of *G. assamicus* are generally exposed to chilling weather during December-March and are vulnerable to death due to freezing temperature (Boorse *et al.* 1998; Pickens & Hester 2011).

Seedling mortality during early stages of growth is very common in many forest tree species. A study in Venezuelan cloud forest showed that 50 percent seedling mortality took place in the first year in two species studied in Flores (1992). In *ex-situ* experiments, we observed foliage damage by insect pests in a few cases and could be responsible for seedling mortality at the early stages. On aging, seedlings establish strong root systems and initiates symbiotic relationships with soil microbes for better growth and survival (Alvarez-Loayza *et al.* 2011). It was found that seedling mortality of *G. assamicus* decreased with the increase in age at Dirang site and could be due to the development of better root system among the aged saplings. Halvorson *et al.* (1991) reported that temperate legume seedlings could fix nitrogen within two weeks of germination. We harvested root of *G. assamicus* seedlings at regular intervals and verified the initiation of nodule formation, however, no such activities could be observed even at the age of two years.

Seedling mortality rate was found much higher at NERIST Botanical Garden compared to Dirang site. This may be primarily due to spatial heterogeneity and substantial change in microclimatic conditions compared to the native *G. assamicus* population site at Dirang. Such differences in seedling growth behavior between two environmental conditions and ability to adapt in new environment depends on the complex interaction of morphological and physiological attributes of each species (Garwood 1996). In the present study, overall seedling performance was found better at Dirang nursery compared to the NERIST Botanical garden, which may be due to favorable microclimatic conditions at the former site. Protection of the seedlings from external human pressure and grazing animals is also important for growth and survival of rare/endangered species (Pare *et al*. 2009). Growth and survival of *G. assamicus* seedlings were found much better in homegardens compared to the open forest areas at Dirang site. Therefore, protection of the seedlings from external damage is very important for successful regeneration of the species. On the other hand, poor growth performance and high mortality of seedlings at NERIST Botanical Garden is mainly associated with water stress and high temperature. Altitudinal range of native *G. assamicus* population is between 1500 - 2100 m asl compared to 110 m as at the NERIST site. Mean monthly temperature was also remarkably lower at its native range. Therefore, differential growth performances and survival of transplanted seedlings at NERIST Botanical Garden could be attributed to the lower altitude and higher mean monthly temperature compared to the Dirang site. Similar result were also reported in the case of *Shorea gardneri* across different light regimes in Sri Lankan rain forest (Ashton 1990).

The most important aspects of successful conservation of rare/endangered species are awareness and involvement of local people (Maschinski *et al*. 2012). Such approach can be more effective in case of plant species having ethnobotanical importance. Since the Monpa people in and around Dirang highly favor *G. assamicus* pods as a soap substitute, we initiated an awareness program among the villagers through village headmen to conserve and reintroduce the species in their homegardens. The response was very positive and many villagers reared the plant species in their homegardens. This helped us enormously during the reintroduction of seedlings and study subsequent growth and survival in and around native distribution range of *G. assamicus* at Dirang.

In conclusion, it was found that survival and growth of *G. assamicus* seedlings were better in moist soil and express full growth and development under medium irradiance level beyond tree canopy. Therefore, plantation along the river or stream bank in moderately open canopy would be the most suitable habitat for successful establishment of the seedlings. Protection of seedlings from insect pest and pathogens at early stages is also important. Although the transplanted seedlings in NERIST Botanical Garden survived during the first few months, long term survival and establishment was significantly reduced during dry seasons of the year. Our *ex-situ* experiments revealed very poor performance of *G. assamicus* seedlings in terms of growth and survival beyond its distribution range. Therefore, reintroduction initiatives should focus in its native range for effective conservation of the species.

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