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RESEARCH PAPER

Regeneration ecology and population status for a critically endangered and endemic tree species (*Ilex khasiana* Purk.) in northeast India

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Abstract: Detailed studies on population structure and regeneration ecology of *Ilex khasiana* were carried out at five representative natural populations i.e. Mylliem (Population – I), Upper Shillong (Population – II), Shillong Peak (Population – III), Laitkor (Population – IV) and Nongpiyur (Population – V) to probe into the responsible affecting factors for its population structure and regeneration ability in these populations. Experimental observation under three controlled light conditions (full sunlight (100%), intermediate sunlight (50%) and low sunlight (30%)) showed that the growth status and survival rates of seedlings under intermediate and low light were better than those under the high light condition. The density-diameter distribution in population of adult trees (≥5cm dbh) exhibited a typical character of a regenerating population. A higher seedling mortality rate in natural populations of *I. khasiana* was most probably due to increase in light intensity following vegetation destruction and other anthropogenic disturbances that cause opening of forest canopy. The findings of the present study would be of immense value in formulating appropriate conservation measures for the species.

Keywords: *Ilex khasiana*; endangered species; population status; regeneration; northeast India

Introduction

About half of the world's vascular plants are highly restricted in their distribution region. The areas of their occurrence have continuously decreased during the last few decades due to various anthropogenic activities (Vega et al. 2004). *Ilex khasiana* is one of the 48 critically endangered species identified world-wide by World Conservation Monitoring Centre (Walter and Gillett 1998) and also is endemic species to East Khasi Hills of Meghalaya in northeast India. Williams (1998) and IUCN (2007) reported that the *Ilex khasiana* was confined only to Meghalaya with a total population size of three to four individuals only. The species was listed under very rare category by Rao and Haridasan (1983) and Haridasan and Rao (1982, 1985). Due to very small population size and its threatened habitats, the species is facing to the risk of extinction.

An understanding of the population status and regeneration behavior is a pre-requisite for developing conservation strategies for the threatened species. Studies on population status and regeneration behavior, which provide vital data on critical stages of the lifecycle of a species, are essential for formulating appropriate conservation strategies (Krenova and Leps 1996). Successful regeneration of a species in nature depends on its ability to withstand disturbance stress that plays a key role in seedling survival and establishment (Rao et al. 1990). This is often achieved through differences in life history strategies and segregation of regeneration niches (Barik et al. 1992). Coppicing is another effective mechanism of tree regeneration following clear-felling and is viewed as an active strategy to withstand dry environment and disturbance of mild intensity (Rundel 1991).

Results of our field survey in northeast India revealed that population size of *Ilex khasiana* was much larger than that reported by earlier researcher (Williams 1998). It is necessary to investigate the causes of poor regeneration in nature. Therefore, the present study was undertaken to: (i) assess the current status of natural population and its regeneration mechanism in nature and (ii) identify responsible affecting factors for constraining the natural regeneration ability, resulting in species decline and (iii) develop a strategy for conservation of the species.

Materials and methods

Study area

The study was conducted in and around Shillong Peak extending up to Mylliem covering an area of about 220 km^2 with an eleva-

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tion range of 1340–1990 m a.s.l. The study area experiences a monsoonic climate with distinct wet and dry seasons. The wet season extends from April to October, followed by a dry period from November to March. During the study period, i.e. 2004-2007, the monthly rainfall during wet season ranged from 306 mm to 2081 mm, while in dry season it was less than 50 mm per month. The average annual rainfall was 5000 mm. The mean monthly temperature varied from a maximum of 26°C in April to a minimum of 5°C in January.

Species description

Ilex khasiana Purk. (Aquifoliaceae) is an evergreen late successional species attaining a height of 15 m. It grows in natural subtropical -broadleaved forest and - pine forest (Champion and Seth 1968) of Khasi Hills of Meghalaya up to an elevation of 1990 m a.s.l (Haridasan and Rao 1985). Flowering starts from May and continues up to July and fruiting takes place during December to February. The fruits are purplish red in colour and 7–8 mm in diameter. The seeds are 3 mm long, oval-ellipsoid or ellipsoid. Field observation indicates that the species requires shade during early stages of growth.

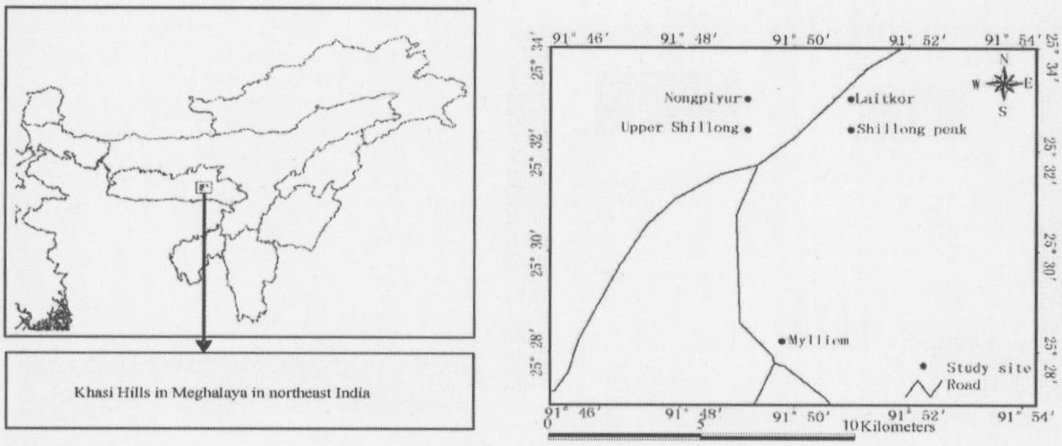

Fig. 1 Study site of *I. khasiana* in Khasi Hills of Meghalaya in northeast India

Mapping of populations

Since the species is endemic to the state of Meghalaya only, the information about the occurrence of the species in different parts of the state was collected from the published literature, flora and herbarium data at Botanical Survey of India, Eastern Circle, Shillong and Department of Botany, North-Eastern Hill University, Shillong, and the local people. A thorough field survey was undertaken in 220 grids of 1 km × 1 km each and the locations of the species were delineated on a Survey of India toposheet. Of these, 170 grids had cultivated fields, roads, human settlements and stone quarries. The rest of 50 grids were surveyed between Mylliem and Shillong peak along north-south extension, and between Upper Shillong and Nongpiyur along east-west extension. The species was found only in twelve localities. Five localities had 19 to 453 adult individuals(≥ 5 cm dbh) and the rest had only one or two individuals. The detailed studies on population and regeneration status of the species were carried out in these five populations viz., Population – I (Mylliem), Population – II (Upper Shillong), Population – III (Shillong Peak), Population – IV (Laitkor) and Population –V (Nongpiyur) (Fig. 1, Table 1).

Population status

Each of the 50 grids was further subdivided into 250 plots of 20 m × 20 m each for detailed enumeration. Of the 50 grids surveyed, *I. khasiana* was found in 50 plots at Mylliem, 14 plots at Upper Shillong, 18 plots at Shillong Peak and Laitkor and 10 plots at Nongpiyur. The population structure and regeneration status of the species were studied by classifying the species into: (1) adult individuals (≥ 5cm diameter at breast height (dbh) measured at 1.37 m from the ground level) and (2) regenerating individuals that include saplings (<5cm dbh and >1m height) and seedlings (<1m height). In each plot, the number of seedlings, saplings and adult individuals of *I. khasiana* were recorded. Disturbance index was calculated for each plot as the ratio between the number of cut stumps and the number of individuals of all species (Rao et al. 1990).

The adult individuals of *I. khasiana* were assigned to five dbh classes (5-15, 16-25, 26-35, 36-45 and >45cm) to analyze the population structure. The status of regeneration of the species was assessed based on the number of seedlings, saplings and adult trees (Sukumar et al. 1992).

Seed germination, seedling survival rate and growth status

The matured fruits of *I. khasiana* were collected in January, 2005 from the natural subtropical forests at Upper Shillong (Latitude 25°32' N and Longitude 91°49' E, altitude 1600 m a.s.l.). The collected fruits of the species were sealed in plastic bags and were brought to the laboratory. Five hundred seeds were sown in rectangular trays filled with garden soils to study seed germination under laboratory conditions at the North-Eastern Hill University Campus, Shillong (Latitude 25°34' N and Longitude 92°54' E, 1450 m a.s.l). Watering at an interval of 3 days was done to prevent desiccation rate. Seed germination rates were recorded up to sixty-five days from the date of sowing beyond which the seeds ceased to germinate. The emergence of radicle was considered as the indicator of germination. One hundred germinated seeds were transferred and grown under three conditions i.e., full sunlight (100%), intermediate sunlight (50%) and low light (30%).

Growth status of the seedlings was monitored over a period of 15 months after transplantation for investigating the survival and growth pattern in three light conditions. In order to compare the mortality pattern of seedlings grown in the natural and controlled conditions, survival pattern of the seedlings under natural conditions was also studied by tagging newly recruited seedlings (2-4 leaved stage) at each site and the fate of the cohort was monitored over a period of 15 months.

Growth performance data of the seedlings were measured in terms of their height, leaf number, leaf area and dry weight by harvesting 10 randomly selected seedlings from the three light regimes. The harvested seedlings were thoroughly washed with water to remove soil particles. Leaf area was measured using a LICOR-3000A leaf area meter (LICOR, Lincoln, Nebraska, USA). Dry matter yield was determined by drying the plant material to a constant weight at 60°C in an oven.

Table 1. Characteristic features of the five populations of *Ilex khasiana* selected for detailed study

Population	Locality	Species	Elevation (m, a.s.l.)	Slope orientation	Slope angle (°)	Past land use in the site	Current disturbances	Total individuals
Population I	Mylliem	*Pinus kesiya*	1792	South-west	20-30	Cultivation	Extraction of NTFP's	2687
Population II	Upper Shillong	*Pinus kesiya, Exbucklandia populnea, Rhododendron arboreum*	1744	South-east	10-20	Mixed broad leaved forest	Human induced forest fire	419
Population III	Shillong Peak	*Lithocarpus dealbatus, Ligustrum robustrum*	1990	South- east	20-30	Mixed broad leaved forest	Extraction of fuel wood, NTFP's and grazing	66
Population IV	Laitkor	*Pinus kesiya*	1824	South-west	10-20	Mixed broad leaved forest	Human induced forest fire	394
Population V	Nongpiyur	*Pinus kesiya*	1748	North	10-20	Pine forest	Human induced forest fire	119

Population	Number of adult individuals	Number of cut stumps	Number of individuals with multiple stems	Number of saplings	Number of seedlings	Total number of individuals of associated tree species	Total number of cut stumps of associated species
Population I	453	37	7	634	1600	209	2
Population II	21	3	1	23	375	410	27
Population III	46	17	5	12	8	523	66
Population IV	70	18	5	120	204	291	9
Population V	19	7	0	60	40	261	21

Statistical analyses

The data on growth performance of the seedlings were subjected to one-way ANOVA (fixed effect model) to test the variations due to differential light conditions. Assumptions of ANOVA were met through Levene's test for homogeneity of group variances (STATISTICA version 6.0, Stat Soft Inc.). Further, pair wise comparison was done by using Tukey's test to find out the relative impact of different light regimes on the seedling growth. The relationship between disturbance and population structure was studied by developing linear regression models using disturbance index as independent variable and the seedling, sapling, adult and total individuals of *I. khasiana* as dependent variable.

Results

Population characteristics

The total individuals of *I. khasiana* including seedling, sapling and adult individuals varied significantly among the populations. The population – I at Mylliem with 2687 individuals of *I. khasiana* had the highest number while the population –III at Shillong peak had the lowest number with 66 individuals of *I. khasiana* only. The population – I had 453 adult individuals, and population –IV, –III, –II and–V had 70, 46, 21 and 19 adult individuals, respectively (Table 1). The species grows in association with pine as well as broad leaved species. At Mylliem, the species was associated with *Pinus kesiya*, which was the dominant species and formed the canopy layer with an average height of 15 m. In this population, the trees of *I. khasiana* also attained the maximum height of 15 m and shared the canopy layer with *P. kesiya*. In Population –II, *I. khasiana* was associated with *Exbucklandia populnea* and *Rhododendron arboreum* which together formed the sub canopy layer. The other associate *Pinus kesiya* was the dominant species and formed the canopy layer. The third population being a regenerating stand had an average tree height of 6 m. The associated ground layer species in all the populations were *Arisaema consanguineum*, *Dricrenopteris linearis*, *Globba clarkeii*, *Imperata cylindrica*, *Oplismenus burmnaii*, *Panax pseudogingseng*, *Rubia cordifolia*, *Rubus* spp. and *Senecio cappa*.

Population structure

The population structure of adult trees (≥5cm dbh) of *I. khasiana* depicted through density diameter distribution yielded a reverse J-shaped distribution curve. All the populations were represented by a few individuals in the higher (>36 cm) dbh classes (Fig. 2). The overall age structures of the population based on densities of seedling, sapling and adult individuals were varied among the five populations (Fig. 3). The highest seedling density of *I. khasiana* (1600 individuals) was recorded in population- I and the lowest in population – III (8 individuals). Similarly, sapling density was also high in population – I (634 individuals) followed by that in population – IV and –V that had 120 and 60 individuals respectively. The relationship between disturbance and seedling, sapling, adult and total population of *I. khasiana* yielded a significant negative correlation (p<0.001), (Fig. 4).

Regeneration pattern

The number of cut stumps was highest in population – I with 37 stumps and lowest in population – II with three stumps (Table 1). The cut stumps were from only two dbh classes i.e. 5–15 cm and 16–25 cm (Fig. 5). Sprouting pattern of cut stumps of the species was common in all the stands, indicating that the species has good coppice capacity (Fig. 5).

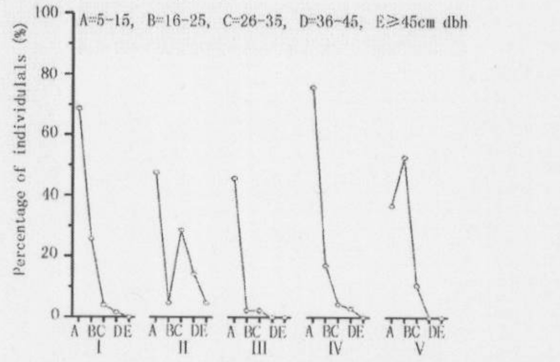

Fig. 2 Density-diameter distribution of adult individuals (≥ 5cm dbh) of *I. khasiana*

Seed germination, seedling survival and growth pattern

About 92% of the total seeds sown germinated under the laboratory conditions. The seed germination started from 23rd day of sowing and continued till 65th day after which no germination was observed. Fifty percent of the germination was observed on 36th day (Fig. 6). The seedling survival percentage ranged from 30% in high light conditions to 92% in low light conditions. Thus, an increase in light intensity decreased the seedling survival rate and also significantly decreased the growth performance (p<0.001) measured in terms of shoot length, root length, number of leaves, leaf area and dry matter yield (Table 2).

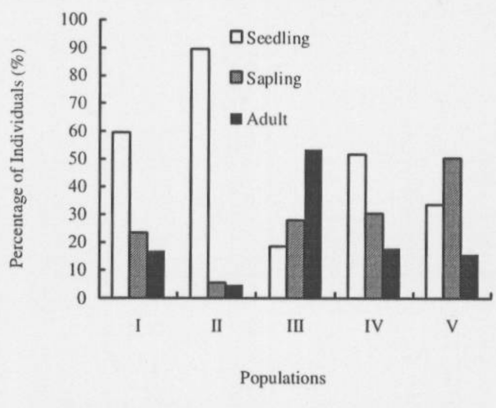

Fig. 3 Population structure of *I. khasiana* in the five studied populations

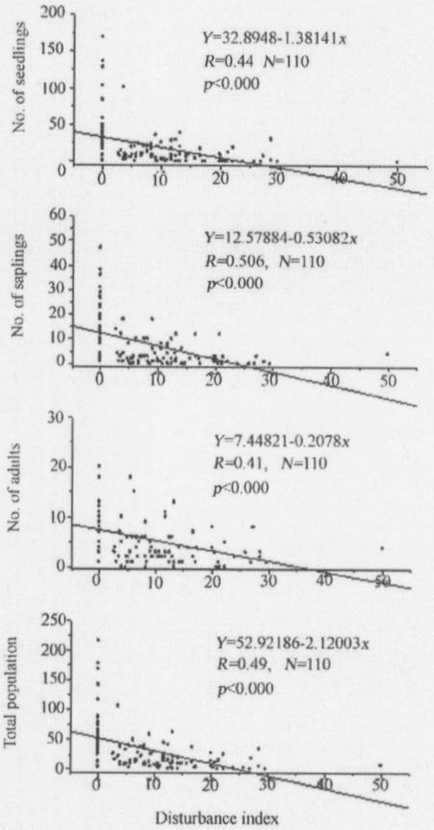

Fig. 4 Relationship between disturbance index and seedlings, saplings, adults and total population of *I. khasiana*

Tukey's pairwise comparison revealed that shoot length, root length, number of leaves per plant, leaf area and dry weight per plant of seedlings growing under the high light regime differed significantly ($p<0.001$), compared with the intermediate and low light regimes. However, the difference in these parameters between the intermediate and low light levels was not significant (Table 2).

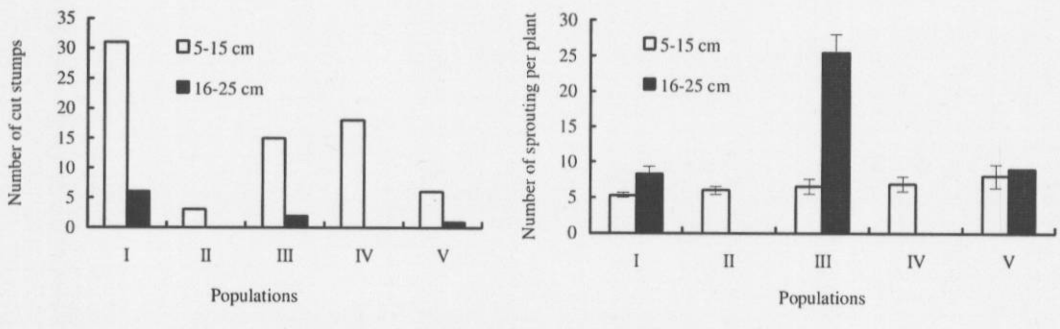

Fig. 5 Number of cut stumps and sprouting/stump in different diameter classes in the five populations

Table 2. Seedling survival rate, shoot and root length, number of leaves per plant, leaf area and dry weight per plant of *I. khasiana* under different light conditions

Light conditions	Survival rate (%)	Shoot length (cm)	Root length (cm)	Number of leaves/plant	Leaf area (cm^2)	Dry weight/ plant (g)
High	30	5.4 ± 0.3^a	9.8 ± 0.5^a	13.8 ± 1.0^a	16.8 ± 2.4^a	0.1 ± 0.02^a
Intermediate	65	7.9 ± 0.4^b	12.6 ± 0.4^b	17.9 ± 0.4^b	40.8 ± 5.4^b	0.3 ± 0.04^b
Low	92	9.9 ± 0.3^b	12.3 ± 0.9^b	19.6 ± 1.4^b	54.6 ± 8.3^b	0.5 ± 0.06^b
ANOVA (F-value)		35.20	16.96	8.36	10.42	20.63

Notes: The values in respective columns are significant at $p<0.001$ (ANOVA). Column with same lowercase letters are not significantly different ($p>0.001$) in Tukey's pairwise comparison of means within the experimental light regimes. "±"----standard error of mean; (n =10).

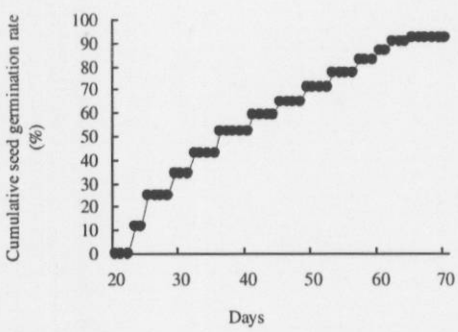

Fig. 6 Germination curve of *I. khasiana* seeds under laboratory conditions

The naturally recruited seedlings exhibited varied survivorship pattern among different populations. Population – I and – IV showed 40% survival rate whereas, population – III had only 14% survival rate. About 45%–84% mortality rate appeared during rainy season (June-September) and winter season (December–February) in all the populations (Fig. 7).

Discussion

The size of the five populations of *I. khasiana* varied significantly (p<0.01) probably due to differences between methods of land uses in the past and present. The populations – I and – II, which were exposed to mild disturbance events due to NTFPs extraction in the community, had significantly larger size than those subjected to more severe and frequent disturbance events characterized by movement of humans, cattle grazing, over-exploitation of biomass and forest fire. The adverse affect of disturbance on *I. khasiana* population was evident from highly significant negative correlation between disturbance index and densities of seedling, sapling and adult trees in the population. Such a disturbance-linked decline in population size of *I.khasiana* was conformed with the findings of Gazoul et al. (1998) and Kadaval and Parthasarathy (2001) who concluded that tree extraction drastically reduced the densities of naturally occurring of flowering trees.

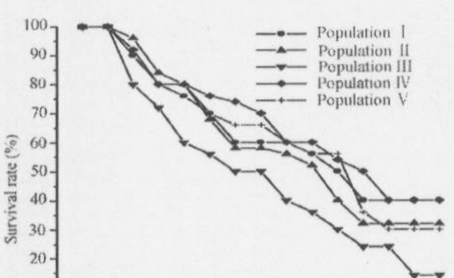

Fig. 7 Survivorship pattern **of *I. khasiana* seedlings in the five studied populations**

The survival and growth performance of *Ilex khasiana* seedlings in low and intermediate light intensities was (p< 0.001) significantly better than that in high light condition. Higher shoot length and leaf number in low (30% sunlight) and intermediate (50% sunlight) light intensities as compared to full sunlight were traits of shade-tolerant species. Non-significant difference in all the growth parameters between low and intermediate light conditions (Tukey's pair wise comparison, p >0.001) further indicates the shade preference of the species. Higher shoot growth rate and greater biomass allocation to shoot are believed to be an adaptive strategy of shade tolerant plants to enhance light interception (Crawford 1989). Therefore, high light intensity in disturbed habitats due to tree cutting seems to be the main responsible factor for poor regeneration and decline in the population size of *I.khasiana* in most habitats.

Natural regeneration of this species through seeds is often impaired by unfavorable environmental condition during the periods of germination and seedling establishment. The fruiting and seed dispersal in this species occurred during winter season (January – March) when dry and cold condition prevailed. The seeds are often induced into a state of dormancy due to unfavourable germination conditions. Some of them are destroyed by the fire set by the local people during this season to clear dried plant debris. As germination conditions become favourable after few showers in the ensuing spring season (April - May), *I. khasiana* seedlings grow along with other plant species. They show poor growth and low survival rate probably due to competition with associated species.

The species also regenerate by coppice, which is an important regeneration mechanism to maintain a viable population in many tree species (Boring et al. 1981; Castillo and Hall 2000; Luoga et al. 2004). Coppicing is a common means of propagation in many species in disturbed habitats with greater light intensity (Rundel 1991). In *I. khasiana*, coppicing takes place mostly in the cut stumps of lower diameter classes (5–15 cm and 16–25 cm). Local people being aware of this coppicing behavior of species preferentially cut the tree trunks of these diameter classes for using it as a pole for construction and other household purposes and leave the stumps for regeneration.

Conclusion

The present study showed that there is much greater population size (3697 individuals) of *I. khasiana* than that in the earlier reports (3-4 individuals) of Williams (1998) and IUCN (2007). The populations at Upper Shillong, Laitkor and Nongpiyur which were growing in relatively less disturbed community owned forest showed better growth and survival rate than the other two populations growing in more disturbed areas. The data generated in the study are therefore crucial for taking up effective conservation measures for the species. Our findings indicate that artificial regeneration methods of propagation need to be adopted to supplement poor natural regeneration for *I. khasiana* populations. The species also needs effective protection mechanism to reduce high seedling mortality rate in the nature.

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