



## JOURNAL

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Ecological and adaptation of Tertiary relic plant of *Tetracentron sinense* with  
Climate change in Bhutan Himalaya

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## Abstract

*Tetracentron sinense* Oliv is the monotypic, tertiary relic, primitive, endemic plant and vesselless angiosperm which has remnant botanical feature in taxonomy. Habitat ecology, regeneration structure and radial growth performance of tertiary relic plant, *T. sinense* was investigated in Lamperi and Nobding. *T. sinense* thrives in humid broad leaved of evergreen and deciduous forest up to transitional zone of mixed conifer in steep slopes, rocky cliffs, stream margin, and roadsides. *Quercus oxyodon* Miq, *Acer campbellii* Hok. et al, *Acer sikkimense* Miq are dominated tree species in *T. sinense* community. The altitude from 2700 to 2800 masl is the prominent ranges of *T. sinense* habitat as ascertained by clustered population predominately with similar content of soil properties and average slope gradient 49% and 76% in average for Lamperi and Nobding. *T. sinense* population does not vary with changing soil properties as investigated in 4 plots in each sites. The paired sampled *t* test confirmed that, there is significant differences in radial growth performance of *T. sinense* in Nobding ( $M = 1.74$ ,  $SD = .664$ ) and in Lamperi ( $M = 1.39$ ,  $SD = .538$ ) with  $t(275)$ ,  $P = 0.00$  and are non responsive to climatic factors due to low sensitivity. Simple linear regression showed that ring width has relatively more association with minimum temperature which revealed that species will not adapt with increasing temperature in climate change scenario. The structure pattern of *T. sinense* in Nobding is unimodal and sporadic in Lamperi requiring immediate conservation action through formulating critical conservation policy due to its remnant botanical feature.

**Key words:** Complacent, ecological amplitude, monotypic genus, ring chronology, sensitive.

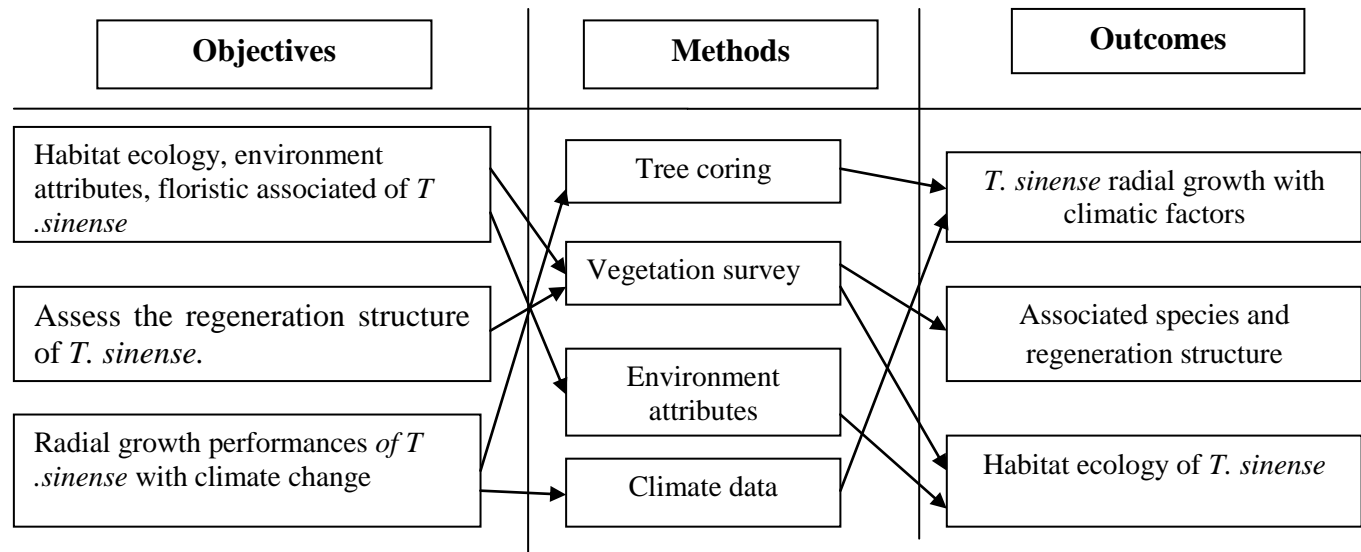
## Introduction

Bhutan is centrally placed in the distribution of the rich Eurasian flora and is home to primitive angiosperms like *Decaisnea insignis* Walter and vesselless primitive angiosperm *Tetracentron sinense* (Oshawa, 2012). The study on habitat ecology, regeneration structure and radial growth performances of tertiary relic plant of *T. sinense* is felt significantly urgent owing to endemic and concerns of future extinction in biodiversity hotspot country. The species is stated as 'rare' in International Union for the Conservation of Nature (IUCN) and listed in Appendix III in CITES (Convention on International Trade in Endangered Species) and only occurs in a few restricted areas in East Asia. The rapid destruction and fragmentation of natural habitats are the principal causes of species extinction throughout the world (Crooks & Sanjayan, 2006). *T. sinense* conservation is of great concern for the world, because of their rarity and their phylogenetic

traits. The environmental factors and ecological interaction results in different forest composition and structure and are assessed through inverse J shape, uni-modal and sporadic distribution for overall condition of forest and species sustainability. The formations of narrow and wide rings in tree make up a specific pattern as a response to environmental factors and anthropogenic causes. Radial growth performance with climatic factors will determine the adaptability of relic species as climatic factors is the decisive role for plant growth, but all species will not record the signal for climate reconstruction due to low sensitivity. As climatic change discussions are on focus everywhere, it is imperative to understand the ecological habitat and growth performances of endemic plant with climatic factors to formulate conservation policy to avoid further extinction from this landscape.

The current research will verify the habitat ecology, environment attributes and its associated floristic diversity, Assess the regeneration structure of *T. sinense* and investigate the radial growth performances of *T. sinense* with climatic factors.

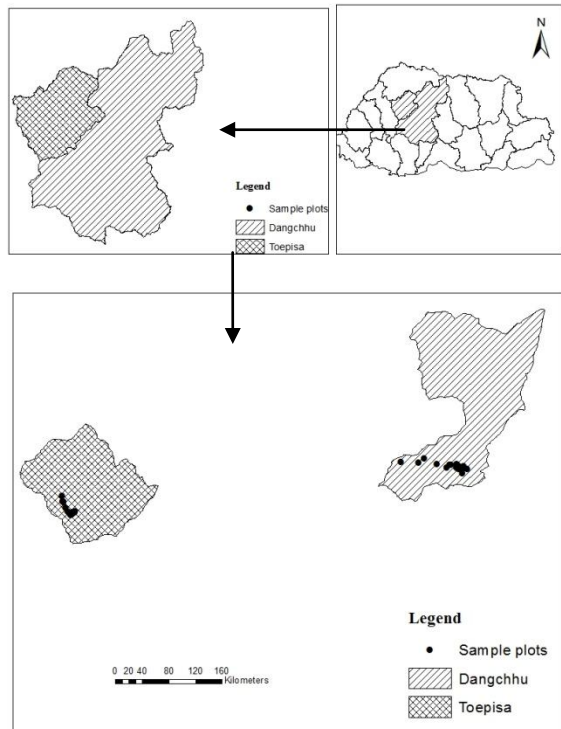
**Conceptual framework model of the study**



**Figure 1.** Conceptual framework model of the study

## Materials and methods

### Study area



**Figure 2.** Map of study area

### Material required

The materials used in the field work are: GPS, Increment borer, Sunto clinometers, Compass, Diameter tape, Measuring tape, Digital hypsometer, Transponder, bamboo tubes, Masking tape,

### Field data collection

Rare plant species are sparsely distributed across the landscape, appearing infrequently and confined to certain range where targeted samplings were deployed.

### Tree coring

Two cores from each tree were done along the contour line with code id C1 (left) and C2 (right) referring from uphill. The parameters like diameter at the base (db), diameter at breast height (dbh), height of the trees (ht) and other factors such as aspect, slope and altitude were recorded.

*T. sinense* is rare and found in Lamperi (2700 m.a.s.l.), Toepisa Geog, Punakha and Nobding (2800 m.a.s.l.), Dangchu Geog, Wangdue which harbors diverse floristic composition including endemic plant species. The site falls in the transitional zone of humid broadleaved to conifer forest zone



**Figure 3.** Extracting core (A) Sample after sanding (B)

#### *Vegetation survey*

Vegetation survey was conducted to examine the dominant species in *T. sinense* community. The plot sites were determined by existence of *T. sinense* stand and correspondingly laid around. Tree diversity (Height  $\geq 1.3$  m) was recorded with DBH and height within quadrates of 20 m x 20 m. Sub plots of 2 m x 2 m in centre were laid to determine regeneration (Height < 1.3 m) and relative abundance of ground vegetation was recorded.

#### *Soil sample collection*

Soil samples were collected by setting small quadrates (0.5 m by 0.5 m) from 4 plots; highest and lowest elevation, clustered and scattered population of *T. sinense* habitat.

### **Data Analysis**

#### *Cross dating for skeleton plotting and Composite plotting*

Core samples were mounted and sanded with increasing grade sand papers and skeleton graph was plotted followed by composite graph to serve as master chronology to other samples cores.

#### *Laboratory Analysis*

Core samples were measured for the annual rings count by using the J2X program. Tree ring sequences were measured under a stereo microscope to an appreciation of 0.001 mm using the computer based travelling stage in laboratory. These annual ring measurement data was verified by using COFECHA software program.

### *Diversity*

Species diversity index ( $H'$ ) was calculated by using Shannon and Wiener Diversity index where the species evenness ( $J'$ ) was calculated using Pielou's method (1969).

$$H' = -\sum_{i=1}^s p_i * p_i \ln p_i - \text{Equation 1}$$

Where;  $P_i = n_i/N$

$$p_i = \text{the relative abundance of each species. } J' = \frac{H'}{\ln(S)} - \text{Equation 2}$$

Volume was calculated by multiplying the height of tallest individual by the percent coverage of each species for ground cover ( $V = \max Ht \text{ in cm} * C \%$ ) - Equation 3

### *Dominance*

The RBA of each species was used as abundance of species and the dominant species in each plot were determined by dominance analysis (Ohsawa, 1984).

$$RD \% = \frac{\text{Volume of individual plant speceis}}{\text{Total volume}} \times 100 - \text{Equation 4}$$

### *Composition*

Composition was analyzed running through pivotal table using species diversity. Composition for both species level and life form were analyzed.

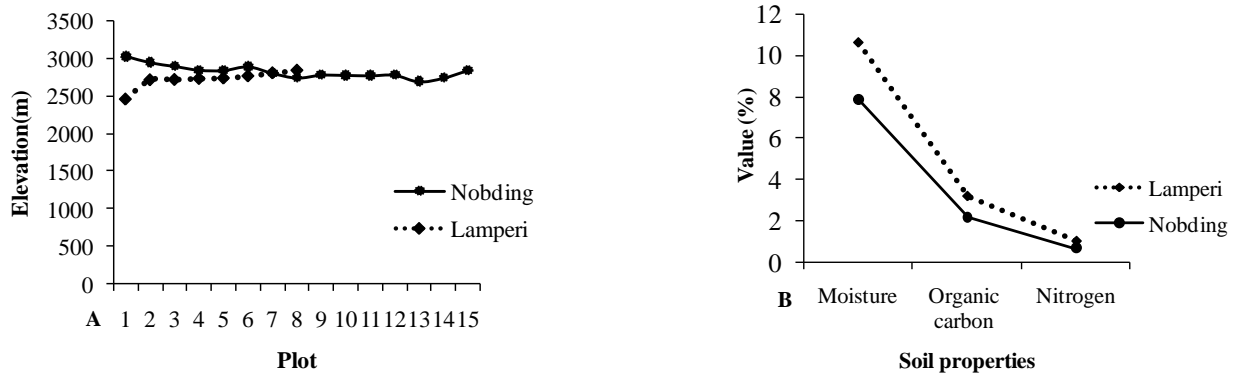
## **Result and Discussion**

### *Habitat of tertiary relic plant T. sinense*

*T. sinense* thrives in humid broad leaved of evergreen and deciduous forest up to transitional zone of mixed conifer in unstable habitats of frequent disturbances, steep slopes, rocky cliffs, stream margin, roadsides, and in bamboo coverage. These are the principal controlling factors of the distribution and survival of *T. sinense* in Bhutan as similar result is also reported by Tang *et al.* (2013) in china. It is light demanding species; appearing to be stress tolerant and their regeneration depend entirely on disturbances with little or no competition from other species (Juan *et al.*, 2008).

### *Environmental attributes*

*T. sinense* appears at highest elevation of 3038 m and lowest of 2696 in Nobding. Similarly, Lamperi has highest elevation record of 2845 m and lowest elevation of 2468 m of *T. sinense* survival. The graph (Figure 4 A) depicts the plots location in two sites, the plots descent from high elevation in Nobding and ascent from low elevation in Lamperi. The altitude from 2700 to 2800 m is the prominent ranges of *T. sinense* habitat. All 8 plots in Lamperi and 11 plots in Nobding face North East aspect. Rigg (1993) point out that, north facing slopes generally have less sunlight and in turn have higher moisture levels and greater vegetation establishment resulting in more organic matter. It was found that, the average slope gradient is comparatively low in Lamperi than Nobding in *T. sinense* habitat area with average of 49% and 76% respectively. It has proven that topography and related edaphic characteristics affect the tree species distribution (Ferreira *et al.*, 2007).



**Figure 4.** Location of study plots in the two sites (A) Trends of soil moisture, organic carbon; nitrogen in two sites (B)

The content of soil moisture and organic carbon is relatively high in Lamperi as depicted (Figure 4B). Comparatively, Lamperi plots with cluster population of *T. sinense* has slightly high soil properties like moisture, organic carbon, nitrogen, pH but low phosphorous than plot with clustered *T. sinense* in Nobding.

There is negative correlation between stem densities of *T. sinense* and nitrogen content, ( $r = -.257, p > .05$ ). Nitrogen content in the soil alone has no significant effect in the population density of *T. sinense* in the plot.

#### **Floristic composition of major life form**

The main companion species of *T. sinense* communities in both sites are *Q. oxyodon* under fagaceae family in evergreen tree life form, *A. Campbellii* and *A. sikkimense* under Acerceae family under deciduous tree life form. *Daphne bholua* Buch *et al*, and *Ilex dipyrena* Rehder are dominated species for the regeneration category in *T. sinense* habitat area. *Sarcococca saligna* Don under buxaceae and *Yushania mycrophylla* Munro *et al*, under gramineae family are the dominated ground cover species in *T. sinense* community area.

In Nobding, a total of 19 tree species belonged to 14 families classified to 4 major life form of conifer; deciduous, evergreen tree and evergreen shrub were enumerated as associated species of *T. sinense*. A total of 10 species belonged to 7 families classified to 4 major life form of evergreen shrub, conifer, deciduous and evergreen trees were recorded in regeneration category. Similarly, ground covers of 14 species belonged to 10 is classified to 5 major life form as evergreen shrub, herb, fern, climbers and bamboo.



In Lamperi, 22 species belonging to 17 families classified to 5 life form of evergreen tree, deciduous tree, conifer tree, evergreen shrub and deciduous shrub were recorded. The regeneration survey revealed a total of 11 species belonged to 9 families under 4 life forms from such as evergreen shrub, evergreen tree, deciduous shrub and deciduous tree. Correspondingly, 24 species belonged to 17 families classified to 5 life forms.

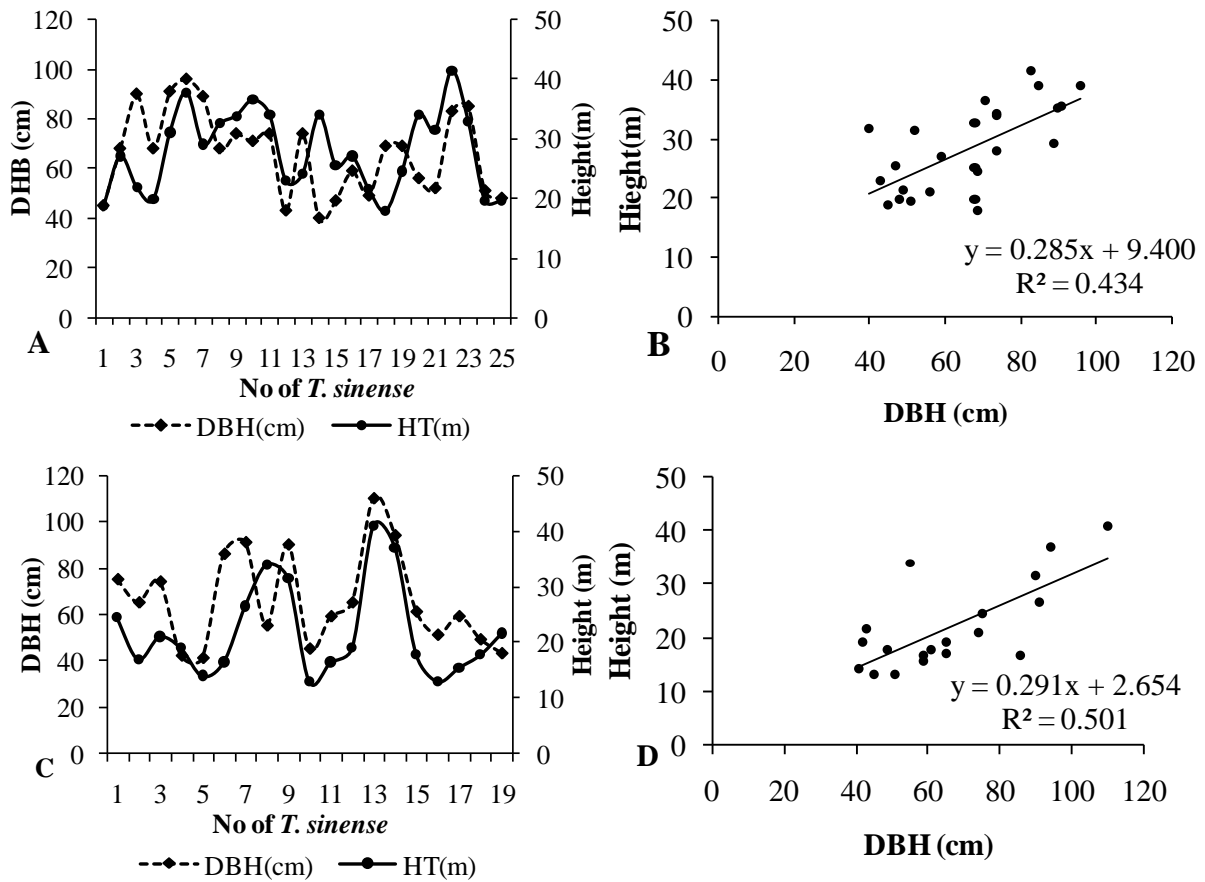
### **Structure of *T. sinense***

#### *Height and DBH*

There is positive association between DBH and height of cored *T. sinense* in both sites. The DBH growth has direct impact on height of tree as the parameters are commonly used to measures of tree growth and size. (Sumida *et al.*, 2012). The relationship established ( $r = .410$ ,  $p < .05$ ) is positively correlated. Simple regression equation, ( $R^2=0.434$ ,  $\hat{y}=0.28x+9.40$ ) showed that only 43% of independent variable of height depends on DBH for its growth and rest 57 % remained unexplained. It further conveyed that when DBH is 0, height is already 0.28 m or increase in one unit of DBH raise height by 9.40 m. (Figure 5 AB) in Nobding.

Similarly, there is positive correlation between DBH and height of *T sinense* in Lamperi where ( $r = .708$ ,  $p = .001$ ). Sumida *et al.* (2012) point out that, relationship indicates that the amount of leaves on a tree was an important factor in DBH growth and then height for the process of photosynthesis. The strength in simple linear regression equation, ( $R^2=0.501$ ,  $\hat{y}=0.291x+2.654$ ) explained that 50% of independent variable height is dependent on DBH and remaining 50% remained unexplained.

The unexplained attributes may be due to influential of environment factors like elevation, soil properties, topography, aspect, slope and competition from neighboring species. The anthropogenic disturbance causes disruption of forest structure and changes community composition which ultimately leads to disruption of tree population structure (Sahoo *et al.*, 2009).

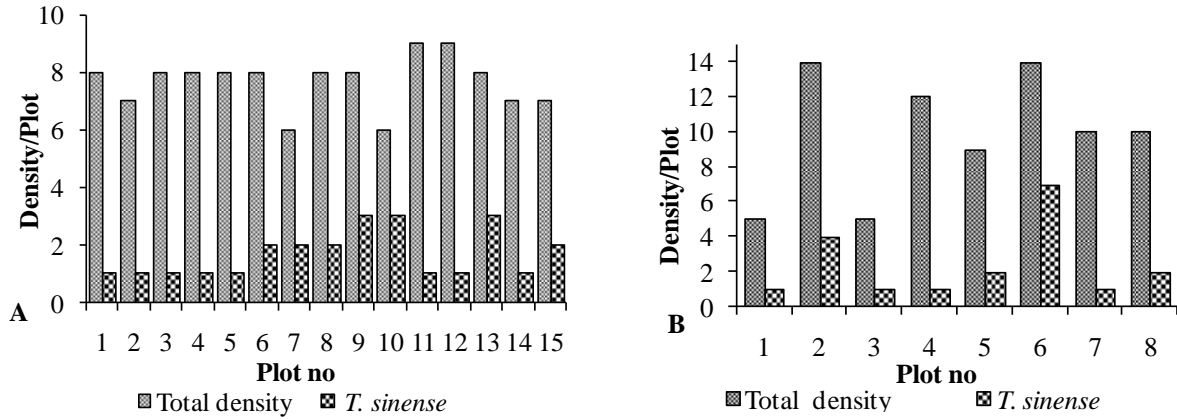


**Figure 5.** Correlation between *T. sinense* DBH and Height for Nobding (A) Simple regression for Nobding (B) DBH and Height for Lamperri (C) Simple regression for Lamperri (D).

#### *Stem density and basal area*

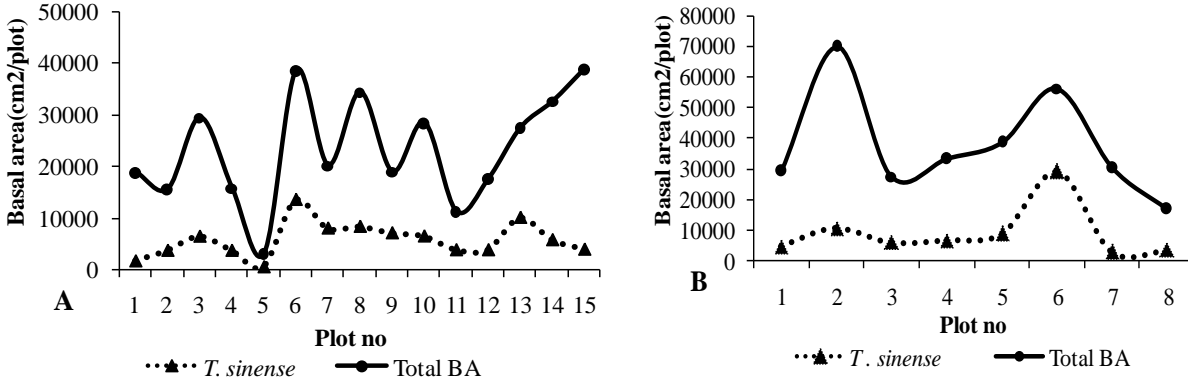
The growth patterns of all trees are not responding homogeneously in two sites. *T. sinense* stem density/plot trend to increase toward descending elevation (within differences of 100 m) with changes in stem density of other associate species in Nobding. There is dramatic variation in stem density of *T. sinense* with other associated species especially in plot no 2, 4 and 7 (Figure 6) for Lamperri. Many study revealed that neighborhood effects on individual tree growth are primarily negative, owing to competition for limiting resources (Burton, 1993)

The overall pattern of stem trends to decline with increase in DHB and height distribution in both study sites as similar finding is also reported by Sahoo *et al.* (2009) reasoning with increase competition.



**Figure 6.** Comparative of stem densities of *T. sinense* with associate species, Nobding (A) Lamperi (B)

The trends of basal area for all plots in both sites are depicted (Figure 7AB). There are variation of basal area among plots due to differences in DBH and competition. Excessive disturbances factor activate to threaten the growth and survival of tertiary relic species than other associated species. The overall average basal area in Lamperi is relatively higher than Nobding as it constitutes more stem density/plot and high DBH.



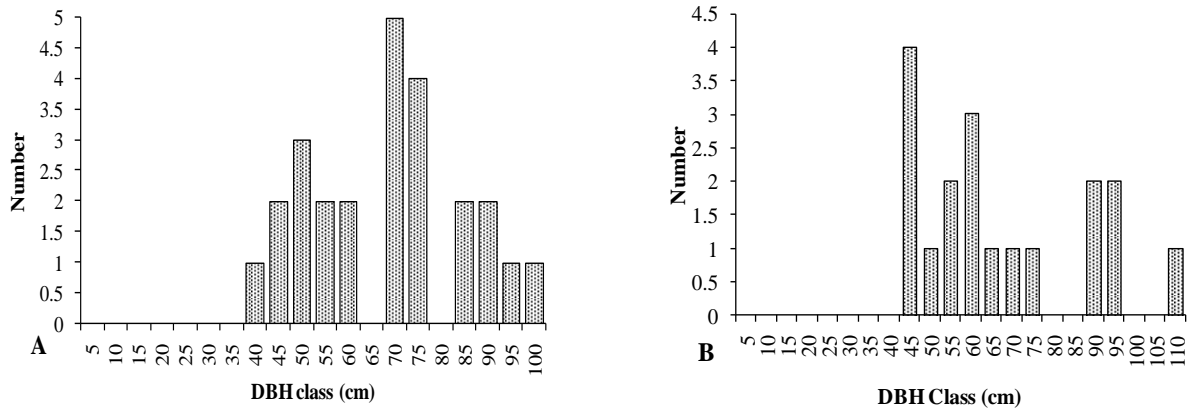
**Figure 7.** Relationship between basal areas of *T. sinense* with associated species, Nobding (A) Lamperi (B)

**Population structure and conservation strategies for relic species**

The population of 44 stems was recorded from two study sites constituting basal area 15.66 m<sup>2</sup>/hectare. The DBH class distribution ranges from 40 - 96 cm and height ranges from 17.8 - 41.2 m. In Lamperi, the highest DBH recorded was 110 cm and lowest was 41 cm for *T. sinense*. Conversely, the highest height was 41 m and lowest height was 13 m.

The chronology series in Lamperi revealed suppressing and releasing in many occasions due to endogenous factors (Figure 11). In both sites, the result portrayed that taxa have restricted in site specific geographical distributions and relatively small population sizes, which predisposes them to a high risk of extinction. During flowering and pollination in natural population, the decrease of population density and harsh environmental condition might be one of crucial reasons resulting in endanger for this species.

The structure pattern of *T. sinense* in Nobding is uni-modal and sporadic in Lamperi site as depicted (Figure 8). The uni-model distribution is indication with comparatively fewer juveniles relative to adults has been taken as evidence of population decline and sporadic type model is due to intermediate disturbance (Wangda *et al.*, 2009). The variation of stand structure is due to differences in micro site factors and disturbance cause as also ascertained by annual mean ring chronologies (Figure 11). Tang & Ohsawa (2002) reported that, ancient relic plant survived in unstable site where there is less or no competition from other species.



**Figure 8.** Population structure of *T. sinense* in Nobding (A) Lamperi (B)

The reason of missing smaller recruitment may be due to grazing, leaving only matured trees in difficult area. Thus, it is pertinent to advocate the significant of endemic taxa not only due to unique in taxonomy, but more importantly, the manifestation it carry along, be it ecological, economic, evolutionary and, genetic diversity in landscape. It is significant to conserve *T. sinense* associated species in their natural habitat. It is vital to necessitate for enlarging its population and individual numbers promptly through artificial intervention as reported by (Lingling *et al.*, 2013.).

### **Regeneration modes of the tertiary relic deciduous trees**

The study found that, there was no regeneration in both sites indicating the risk of future sustainability. The competitions from associated growing species further narrow the possibility of regeneration. *T. sinense* is pioneer species characterized by long life span with minutes wind dispersed seed as reproduction happen unstable habitat, steep terrain and disturbance sites lose the ability to profuse germination. The harsh environment factor for species habitat and low density of population during flowering and pollination is critical factor for making the poor or no regeneration traits of *T. sinense*.

Rare plant exhibit slightly higher self compatibility than common plant and lack of pollinator facilitate inbreeding depression and attributing for further rare and endangered species. (Saunders & Sedonia, 2006). It is reported by Xiaohong *et al.* (2013) that, pollinator insect for *T. sinense* exhibit nectaring behavior and not the potential pollinator. Establishment of forest species in any site depends on the ability of their seed to disperse, to germinate and to compete to survive (Cavallin & Vasseur, 2008).

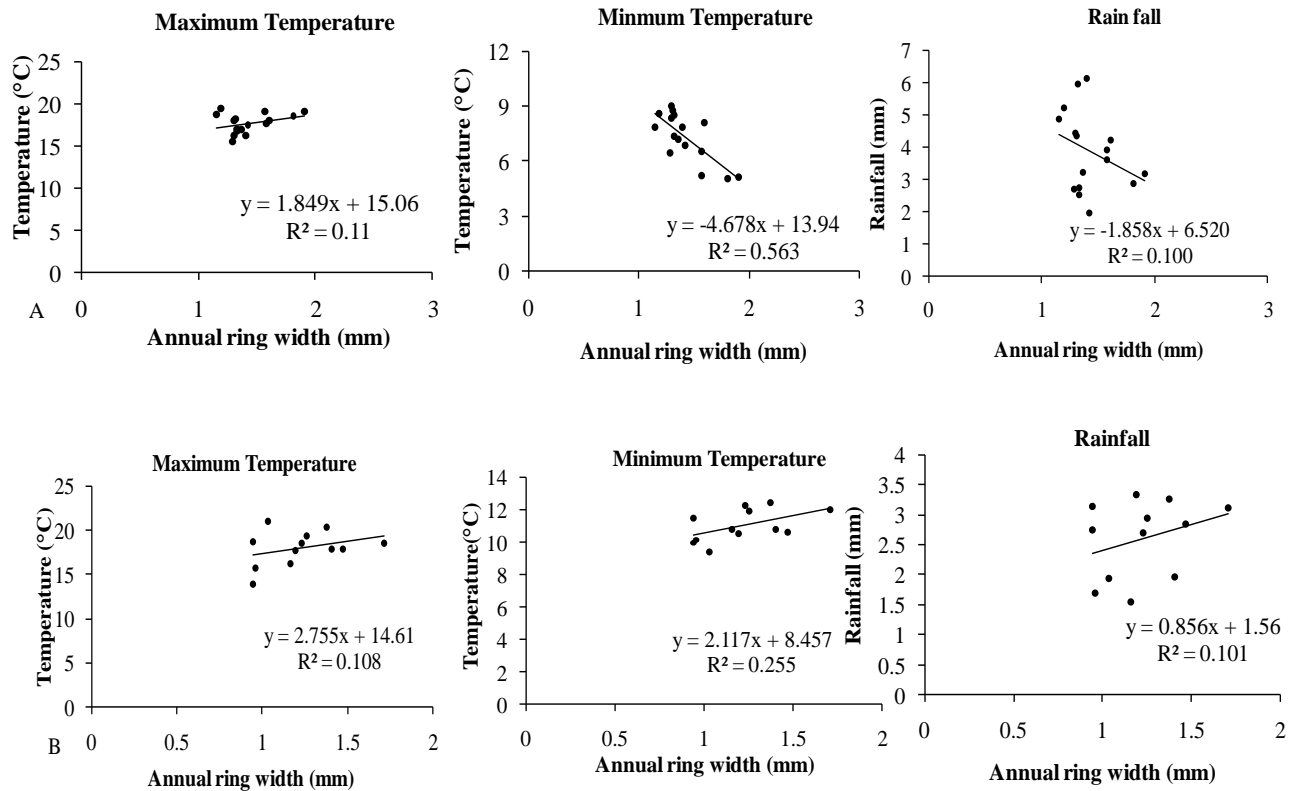
### ***T. sinense* growth response to climatic factors**

Annual ring width series was compared with available climate data from 1996 to 2011 for Nobding and 2004 to 2015 for Lamperi which is very short for climate related studies. The paucity of long meteorological records in Asia has been a matter of concern in the field of dendroclimatology (Cook *et al.*, 2013).

Pearson's rho correlation indicates, *T. sinense* ring width is negatively correlate with climatic factors. However, annual mean ring width showed positive correlation with minimum temperature ( $r = -.751, P < .01$ ) in Nobding. It clearly informs that *T. sinense* does not respond or does not record the climate signal. For climate tree relationship investigation, proper choice of old/mature and climate signal tree is essential (Fritts, 1976), but *T. sinense* being rare, all available population were examine through coring attributing for non signal of climatic factors. Hughes *et al.* (1982) also noted that radial growth responses may vary significantly with climatic, site conditions and the inherited capabilities of tree species.

In Nobding, Annual ring width and minimum temperature has highest relation ( $\hat{y} = 4.67x+13.94, R^2 = 0.563$ ) followed by minimum temperature in Lamperi ( $\hat{y} = 2.117x+8.457, R^2 = 0.255$ ). Thus, result revealed that, *T. sinense* have relatively more association with mininum

temperature in both sites than other climatic variable, suggesting that growth of *T. sinense* is hinder with increasing temperature. Increase temperature under climate change might increase evapotranspiration and reduce soil moisture, further limiting plant growth (Yang *et al.*, 2003) and decreasing forest productivity, which is true for endemic *T. sinense*. Hollinger (1992) revealed that sufficient but not excessive heat is basic prerequisite for plant life.

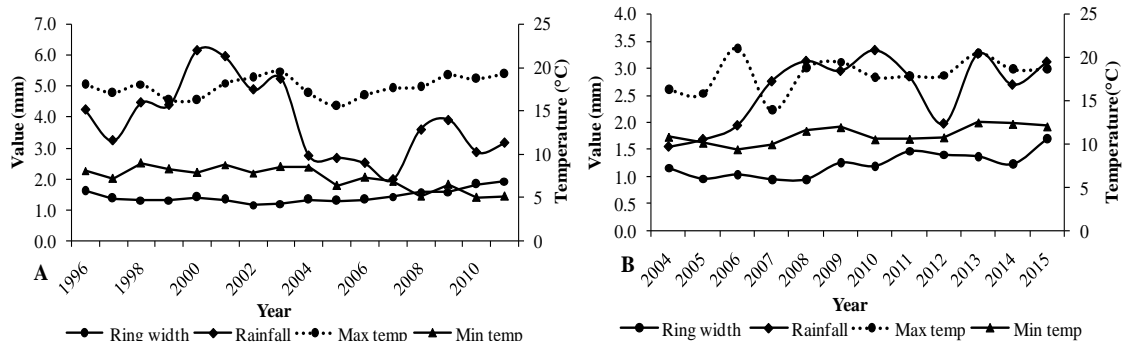


**Figure 9.** Simple linear regression between annual ring width and climatic factors for Nobding (A) Lamperi (B)

*The influence of temperature on radial growth of T. sinense*

*T. sinense* radial growth does not appear to have responded to the variation in temperatures, although it is not clear to draw result with limited climate data at one time study. There are many increases and decrease of radial growth with similar ranges of temperature such as 1998, 2001, 2002 and 2010 but ring width varies from 1.15 – 1.81 mm.

Whilst some higher temperatures coincide with years of lower growth and contrary, but it is not consistent. However, the trends does not appear to be clear with variation of temperature level with no significant relationship being discovered, this research coincides with Rebecca (2014) whose research showed minimal responsiveness to temperature. Way and Oren (2010) suggest increased temperatures increase radial growth, which does not particularly coincide with *T. sinense* radial growth.



**Figure 10.** Annual mean ring width with climatic factors

#### *The influence of precipitation on radial growth*

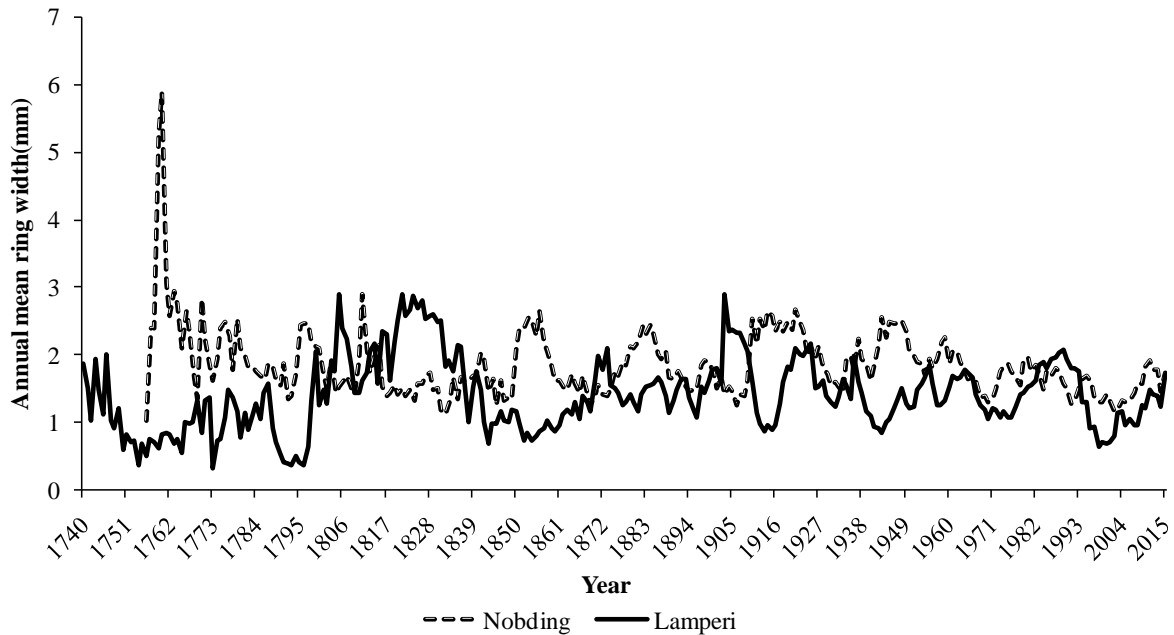
The annual precipitation levels had little or no effect on *T. sinense* radial growth. Highest rainfall of 6.13 mm measured 1.40 mm ring width in 2000 and lowest rainfall of 1.96 mm measured 1.42 mm ring width in 2007. Equally, some of the largest ring widths coincided with years of low precipitation and contrary. Thus, it is hard to interpret as the trend appears is inconsistent. This does not coincide with research from various studies, which imply those years with low precipitation levels result in narrow rings (Bouriaud *et al.*, 2004).

Although the climatic conditions are recognized as a major influencing factors affecting radial growth (Fritts, 1965), there still remained contradiction amongst research from different studies throughout the world related to many different species. The relationship between ring width and climate is complicated by variety of non climatic factors (Fritts, 1976). Aside than climatic factors, tree age, phytosociological position of tree, site differences and disturbance regimes also influence the radial growth, which may hold true for *T. sinense*. The site specific factors such as topography, soil type, forest thinning and ecological parameters like pest infestations on trees can modify the climate ring width relationship (Manage, 1997).

### Mean comparison of annual ring series in two sites

The growth patterns from annual mean ring width measurement in two sites are varied. The paired sampled  $t$  test confirmed that, there is significant differences in radial growth performance of *T. sinense* for Nobding ( $M = 1.74$ ,  $SD = .664$ ) and Lamperi ( $M = 1.39$ ,  $SD = .538$ ) with  $t(275)$ ,  $P = 0.00$ .

The result obtained from dated sample of 31 from Nobding showed 0.127 inter correlation series with average mean sensitivity of 0.261 and means annual length series of 150.3. Correspondingly, 13 dated samples from Lamperi showed 0.141 inter correlation series with average mean sensitivity of 0.300 and mean annual length series of 161.0. The weak intercorrelation among dated sample is attributed by differences in topographic gradients and micro site characteristic between plots.



**Figure 11.** Comparative of annual mean ring width from two sties

Mean annual ring width from 1740 to 2015 (275) old year of *T. sinense* in Lamperi and from 1756 to 2015 (259) old year in Nobding was compared in (Figure 10). The ring pattern of *T. sinense* growth in Nobding is low degree of variation (complacent ring) and high degree of annual variation (sensitive ring) in Lamperi. The rampant disturbances in Lamperi might have attributed for disturbing factor for releasing and suppressing the ring patterns over the years. There is history of anthropogenic and similar finding is also reported by Wangda (2006), where



maximum height of tree showed slight decrease due to gradual sprawling into surrounding forests by increased human disturbance in Lamperi.

The population of *T. sinense* and associated species per plot is relatively higher in Lamperi fluctuating even within the plots which enhance competition among the species, over the years, annual precipitation is comparatively low in Lamperi than Nobding, thus ring formation is wider and narrow. In contrast, habitat in Nobding is restricted to undisturbed area of steep slope in inaccessible area along the margin of stream and in valleys with good water table for moisture complacency with complacent ring formation over the years.

Coder (1999) reported that, water supply accounted for around 80% of variation in ring growth. Water is the most limiting ecological resource for most tree and forest sites. Tree ring response to climate is not necessarily limited to the growing season, but rather to the most influential conditions of water availability, which the tree may be put under (Hughes, 2002).

## **Conclusion**

Geographically, *T. sinense* is restricted species in cool humid evergreen and deciduous forest to transitional zone of mixed conifer in Bhutan. The main dominated associated tree species of *T. sinense* are *Q. oxyodon*, *A. campbellii*, *A. sikkimense* based of relative basal area.

The study showed no single regeneration and distribution pattern was uni-modal and sporadic structure for Nobding and Lamperi. *T. sinense* exhibit non response to climatic factors due to low sensitivity, but relatively respond with minimum temperature. Therefore, it signifies that relic species will not adapt with increasing temperature in climate change scenario. The radial growth pattern of *T. sinense* results significant differences between two sites with complacent and sensitive ring formation which is attributed by disturbance regimes and other environmental factors. Sustainance of it genetic diversity is uncertain as the current trends of population structure is declining by habitat destruction coupled by low reproduction rate. In order to preserve the ancient genetic diversity of this phylogenetically distinctive and increasingly threatened species, protection of companion species in their habitat and artificial measures are crucial to revive the declining species.

There was no information about the area coverage and distribution pattern of relic species precisely in advance, systematic sampling could not be done and the study was done through targeted sampling. Lacks of long climate data hamper to relate the annual ring width for longer

time scale. The findings of the study are based entirely on one time data collection confined in two study sites, and therefore the findings may not necessarily be generalized and apply in global context. Since, the smaller populations are rare, it is very important to clarify the cause of absence of recruitment of *T. sinense* through detail research with setting permanent observation plots for this species habitat in future.

### **Acknowledgement**

The author would like to thank Rufford Small Grant Fund based in UK for generous financial support, of which project would not have materialized without funding

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