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DETERMINATION OF GROWTH PERFORMANCE OF MANDARIN (*CITRUS RETICULATA* BLANCO) IN RELATION TO SURROUNDING CHIR PINE (*PINUS ROXBURGHII* SARG.) AT DAGANA DISTRICT THROUGH DENDROCHRONOLOGICAL ANALYSIS

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Abstract

Tree-ring growth is closely related to climate and the environmental changes during the growth period; thus, it can record precise information on climate variations. This study aimed to understand changes in radial growth and develop tree-ring chronologies of *Citrus reticulata*, validated by tree-ring chronologies of *Pinus roxburghii* within the same micro-site. Tree-ring data were obtained from the sample cores collected from the two different altitudinal sites (1,150 to 1,250 masl) 400 to 500 m apart and compared the climate data from Drujeygang meteorological station. Using dendroclimatological methods, summary statistics were obtained through cross-dating and standardization, and tree-ring chronology was developed. Ring-width chronologies spanning 36 to 41 years were developed for *C. reticulata* and 57 to 174 years for *P. roxburghii*. One-way ANOVA test analysis showed spatial patterns of variability within the altitudinal range of 1,150 to 1,250 masl. Correlation analysis indicated that the growth of *C. reticulata* was highest at the mean annual temperature range of 19.0 to 19.4°C and mean annual precipitation range of 86.51 to 147.45 mm. *C. reticulata* showed a positive relationship with mean annual temperature and precipitation, and in contrast, *P. roxburghii* showed a negative relationship. However, both *C. reticulata* and *P. roxburghii* in both regions depicted a remarkable variability in growth due to fluctuating climatic factors. This study evaluated the relationship between tree-ring growth and climate factors and is expected to contribute to the understanding of changes in citrus productivity in the region and its relationship with climate change in the future.

Keywords: *Citrus reticulata*, climate change, climate growth response, dendroclimatology, tree-ring growth

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Introduction

Mandarin (*Citrus reticulata* Blanco) is considered to be the true citrus (Ezeabara and Okeke, 2016) of the three true species under the family Rutaceae (subfamily Aurantioideae and tribe Citreae) (Phetkul et al., 2013; Ikawati et al., 2019). It is also known to be a single variety amongst the most varied groups of citruses consisting of several genera and interspecific varieties, often leading to difficulty in the classification. It is considered to have originated from China, India, and Bhutan (Dorji and Yapwattanaphun, 2015), where diverse species exist in the wild, established orchards, and also in backyard farms mostly grown from seeds.

It is one of the most popular horticultural fruit crops currently grown in over 140 countries in the world (Dorji, 2011) and 17 of the 20 districts in Bhutan (Dorji et al., 2016; Ghosh, 2020). Currently, citrus is reported to be growing within the range of 300 masl at Sunkosh in Dagana to over 1,850 masl at Wengkhar in Mongar district. It is predominantly grown in Dagana, Sarpang, Tsirang and Zhemgang districts of Bhutan (Ghosh, 2020), in which these trees are seen overlapping with the mixed chir pine and broadleaved forest growing range (900 to 1,800 masl) (Mukhia, Wangyal and Gurung, 2011). This portion constitutes 2.50% of the pure chir pine forest in Bhutan (Mukhia, Wangyal and Gurung, 2011).

Over 60% of the rural population in Bhutan depends on mandarin cultivation with an annual production of more than 72,000 tons from an approximate area of 4,500 ha (Dorji and Yapwattanaphun, 2011). More than 60% of the total annual production is exported to the neighboring countries of Bangladesh and India. Commercial Mandarin cultivation has significantly contributed to income generation for local subsistence farmers and for the government as a whole.

However, decreasing citrus trees within a few years of plantation or orchard establishment is a foremost concern being faced by citrus cultivars all over the country (Dorji, 2011). Generally, various pests and diseases of citrus were studied, but tree-ring scientists have normally ignored the use of dendrochronological techniques to assess the growth and impacts of climate change on major horticultural trees, including mandarin (Abrams and Hock, 2006).

Moreover, extensive research has reported that due to external shocks like heat stress change, drought, CO_2 assimilation, intensive rainfall, and relative humidity, climate change will impact on subsistence farming which could be the main challenge in the future (Chhogyel and Hasan, 2020). A simulation model reported by the National Environment Commission (NEC) had shown great irregularity in both the distribution and pattern of rainfall in the country and the region as a whole (Chhogyel and Hasan, 2020). Such climatic constraints lead to the production of tree rings in citrus (Abrams and Hock, 2006) and the growth response to climatic variation can be investigated from studying the differences in radial growth.

Mandarin being one of the major export commodities of Bhutan, it is important to study its response to climate change through dendrochronological techniques along with diseases and pest control methods to ensure the sustainability of citrus production. The ring growth is directly affected by the exposure to different atmospheric conditions, such as moisture, temperature, sunlight and environmental stress. This study will help to understand better about the current environmental processes and conditions, and improve the overall understanding of possible environmental problems for better growth. Dendrochronology has a remarkable and underexploited power to understand the factors driving the complex climatic systems (Roloff, 2014; Moon et al., 2020) often providing precise key information about how to manage them. Only a few studies have been done and very little is known about its response to climate change, habitat, and management. Hence, this study aimed to determine radial growth response in relation to climatic factors and assess the climatic impact on the growth performance and distribution of *C. reticulata*.

Materials and methods

Study area

The study was conducted in Dagana, a west-central district of Bhutan that shares its district boundaries with Thimphu and Chukha to the west, Tsirang to the east, Wangduephodrang to the north (Figure 1), and the Indian border to the south. According to the Dagana District Administration [DDA] (2018), it comprises 14 Gewogs (administrative block) with two agro-ecological zones: a temperate zone in the north and a sub-tropical zone in the south, covering an approximate area of 1,389 km^2. The region experiences hot and wet summers and dry, cold winters. The western half of the Phibsoo Wildlife Sanctuary also falls under this district. The district constitutes over 80% forest coverage (DDA, 2018), and *Shorea robusta* and *P. roxburghii* are found in the district.

The district lies within the elevation range of 600 to 3,800 masl (DDA, 2018) and the majority of the district area experiences a warm and humid climate, while the northern regions experience moderately cool temperature. The average annual rainfall ranges from 1,000 to 2000 mm receiving the maximum amount during July and August. The district's population is primarily dependent on dry-land agriculture (Kamzhing), with a smaller number also engaging in wet-land agriculture (Chhuzhing). Mandarin is one of the main cash crops of the district with orchards established in all the Gewogs (administrative block).

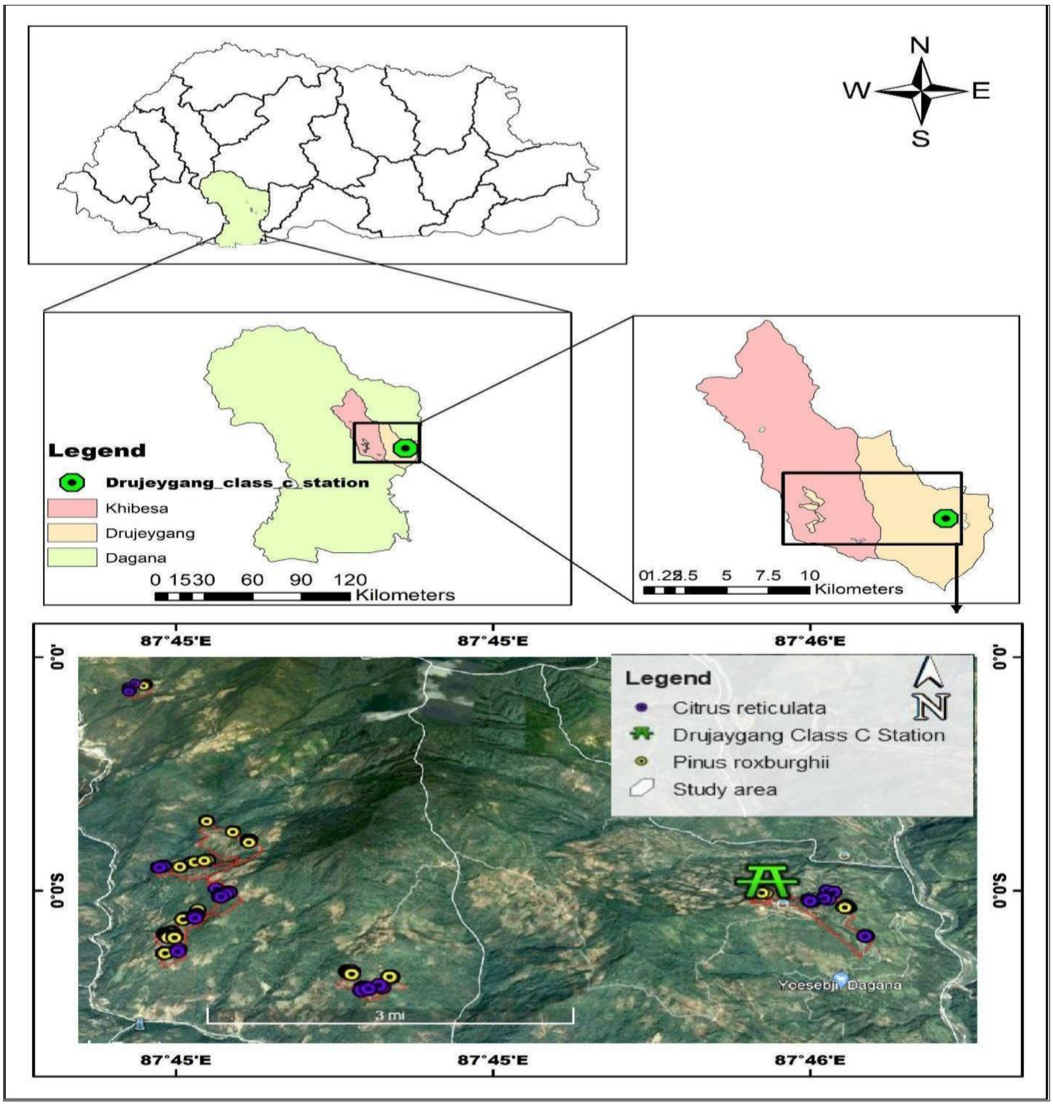

Figure 1: Study area of the *C. reticulata* and *P. roxburghii* in Dagana

After consulting with the district agriculture officer and orchard owners, this study selected orchards in two Gewogs: Kebisa and Drujeygang, with an altitudinal variation from 200 to 300 m. Sample cores were collected from both of these regions covering, 110 ha and 90 ha from the upper and lower sites, respectively, as these Gewogs are important for mandarin cultivation within the district. These two Gewogs lie within altitudinal ranges of 650 to 2,350 masl for Khebisa and 350 to 2,250 masl for Drujeygang. Both areas consist of chir pine forests and citrus orchards, as required for this study.

Sampling method

The sample collection sites were selected based on the concept and principles of site selection, with a focus on areas most likely to exhibit tree stress and significant growth variability. Tree sample cores were collected from two regions using a purposive/targeted sampling method, specifically targeting orchards that have been established for a minimum of 20 years. This study considered orchards facing south, south-west, and south-east aspects, located within the altitudinal range of 900 to 1,500 masl.

To obtain the longest possible dendrochronological records, the oldest-appearing and relatively the healthiest trees in the most climatically stressful sites were sampled, recognizing that not all trees record the same signal. Additionally, for the very old and dead *C. reticulata*, three stem disks from both upper and lower sites were collected from the base of the tree. However, *C. reticulata* had only a few decades of data records. In such cases, *P. roxburghii* trees growing within the same site as the sampled *C. reticulata* trees were cored to facilitate climate data cross-dating and to acquire climatic variability data extending further back in time.

Using an increment borer with a standard core diameter of 12.0 mm, two sample cores were extracted from *C. reticulata* trees at a height of 15 cm above the ground. For *P. roxburghii* trees, two sample cores were collected at a height of 1.3 m using an increment borer with a standard core of 12.0 mm. This selection of core diameters aimed to minimize any adverse impact on the sampled trees. For trees located on sloped areas, sample cores were taken from the upper portion of the rootstock parallel to the contour line, and for those trees located in the flat area, sample cores were taken from random locations around the upper portion of the rootstock, while maintaining a 90° angle with respect to the tree trunk.

Core mounting and sanding

The sample cores were properly air-dried and mounted on a wooden grooved block with the xylem vessels oriented in a vertical position. These cores were left to dry for about 24 hours and then sanded. The sanding process involved using increasingly fine sandpaper, progressing from 80 (177 to 210 µm) to 220 grits (57 to 74 µm). Both belt and circular sanders were used, and for better visibility of the tree-ring patterns, some hand sanding was also done.

The ring-widths of C. *reticulata* showed considerably wider early-wood compared to the late-wood, a distinction that required careful observation under the microscope to discern. Any potential presence of false rings was rectified with careful cross-dating with other samples and with the examination of stem cross-sections.

Tree-ring measurement

The tree-rings were counted and measured under a stereoscope using the measure J2X software program. Annual rings were measured from the bark to the pith.

Data sorting and analysis

Data sorting was done in Microsoft Excel. SPSS version 25 was used to run linear regression and correlations. MS Excel was also used to obtain scatter plots and line graphs for both species in both regions. A total of 34 living trees of *C. reticulata* and 21 living trees of *P. roxburghii* were cored from the lower study site, resulting in 68 and 42 sample series, respectively. However, only 21 trees with 42 sample series of *C. reticulata* and 15 trees with 31 sample series of *P. roxburghii* were included in the analysis. To compare the radial growth performance along different altitude levels for both *C. reticulata* and *P. roxburghii* in both the upper and lower study sites, a one-way ANOVA test was performed.

Results and discussion

Tree growth variation

Tree ring-width chronologies were developed for both *C. reticulata* and *P. roxburghii* in both the upper and lower study sites, separately, to observe distinct growth patterns. Both the upper and lower sites showed distinct bands in ring-width patterns. The mean chronologies for the upper and lower sites were 31 years for *C. reticulata,* and 67 years for *P. roxburghii.* In contrast, for the lower site, the mean chronologies were 24 years for *C. reticulata,* and 41 years for *P. roxburghii*, respectively.

Summary of upper-site C. reticulata and P. roxburghii tree-ring index

A total of 87 living trees of *C. reticulata* and 50 living trees of *P. roxburghii* were cored from the upper study site, resulting in 174 and 100 sample series, respectively. However, after cross-dating using COFECHA, only 26 trees with 52 sample series of *C. reticulata* and 37 trees of 74 sample series were included in the analysis, as they showed an overall inter-series-correlation of 0.190 ($p < 0.05$) and 0.204 ($p < 0.05$), respectively. The remaining 148 samples were excluded from the analysis, as they did not cross-date well, and inter-series-correlation was insignificant. The statistics for the characteristics of the standard chronologies for both species are presented in Table 1.

The average measured tree-ring growth was 0.980 mm with a standard deviation of 0.287 for *C. reticulata* and 1.007 mm with a standard deviation of 0.145 for *P. roxburghii.* This means that *C. reticulata,* despite being older, showed relatively smaller tree-ring growth compared to *P. roxburghii*. The statistics used to test normality, kurtosis and skewness, were 0.197, 0.857 for *C. reticulata* and 0.151, 0.228 for *P. roxburghii*, respectively. The mean sensitivity, which measures the sensitivity to changes in climatic and environmental factors, was 0.477 for *C. reticulata* and 0.300 for *P. roxburghii,* respectively.

The values of the Expressed Population Signal (EPS) were 0.59 and 0.53 for *C. reticulata* and *P. roxburghii*, respectively. While it is typically considered to have a significant statistical confidence in an index chronology when the EPS is above 0.85 (Briffa and Jones, 1990), the National Institute of Forest Science

(2016), suggests that for a chronicle covering a relatively wider area, it can be appropriate to use lower EPS data for index chronology. Hence, it does not appear inappropriate to apply the EPS estimations as data for a tree-ring chronology, even though the EPS estimates for both species were relatively low in this study.

Table 1: Basic statistics on index chronology of upper-site *C. reticulata* and *P. roxburghii* tree-ring growth

Sl. no.	*Basic Statistics on index Chronology*	*C. reticulata*	*P. roxburghii*
1		1985-2021	1847-2021
2	Common interval	2004-2021	1999-2021
3	Number of radii	52	74
4	Mean sensitivity	0.477	0.3
5	Standard deviation	0.287	0.145
6	Expressed Population Signal	0.59	0.53
7	Inter-series-correlation	0.19	0.204
8	Mean of the above	0.98	1.007
9	Kurtosis	0.197	0.151
10	Skewness	0.857	0.228
11	Sample variance	0.082	0.021

Summary of lower-site C. reticulata and P. roxburghii tree-ring index

Only 21 trees with 42 sample series of *C. reticulata* and 15 trees with 31 sample series of *P. roxburghii* were used for the analysis, having an inter-series-correlation of 0.238 and 0.230 ($p > 0.05$), respectively. The remaining 37 samples were rejected due to poor cross-dating and low inter-series correlation. The statistics for the characteristics of standard chronologies of both species are shown in Table 2.

Table 2: Basic statistics on index chronology of lower-site *C. reticulata* and *P. roxburghii* tree-ring index

Sl. no.	*Basic Statistics on index Chronology*	*C. reticulata standard*	*P. roxburghii standard*
1	Chronology time span	1980-2021	1964-2021
2	Common interval	2006-2019	1996-2021
3	Number of radii	42	31
4	Mean sensitivity	0.475	0.322
5	Standard deviation	0.294	0.279
6	Expressed Population Signal	0.8	0.66
7	Inter-series-correlation	0.238	0.23
8	Mean	0.962	1.081
9	Kurtosis	-0.2	2.949
10	Skewness	0.424	1.197
11	Sample variance	0.086	0.078

The average measured tree-ring growth was 0.962 mm with a standard deviation of 0.294 for *C. reticulata* and 1.081 mm with a standard deviation of 0.279 for *P. roxburghii*. This means *C. reticulata* with higher age had a relatively small tree-ring growth compared to *P. roxburghii*. Similarly, the normality tests such as kurtosis, and skewness were 0.200 and 0.424 for *C. reticulata* and 2.949 and 1.197 for *P. roxburghii*. The mean sensitivity for *C. reticulata* was 0.475 and 0.322 for *P. roxburghii*. The values of EPS were 0.80 and 0.66 for *C. reticulata* and *P. roxburghii*, respectively.

Radial growth performance of C. reticulata on altitude levels

When mean annual increments of *C. reticulata* in relation to altitude levels were compared, the influence of altitude does not have a significant impact on *C. reticulata* growth ($F(1, 50) = 0.819$, $p = 0.183$) (Table 3), however, graphical representation shows an increased number of mean annual increment above the optimum range (1 mm) within the altitudinal range of 1,150 to 1,250 masl, which was not noticeable in the tree-ring record (Figure 2).

Table 3: Radial growth performance of *C. reticulata* by altitude levels

Sl. no.	***Basic statistics***	***SS***	***df***	***MS***	***F***	***p***
1	Between Groups	0.001	1	0.001	1.819	0.183
2	Within Groups	0.029	50	0.001		
3	**Total**	**0.03**	**51**			

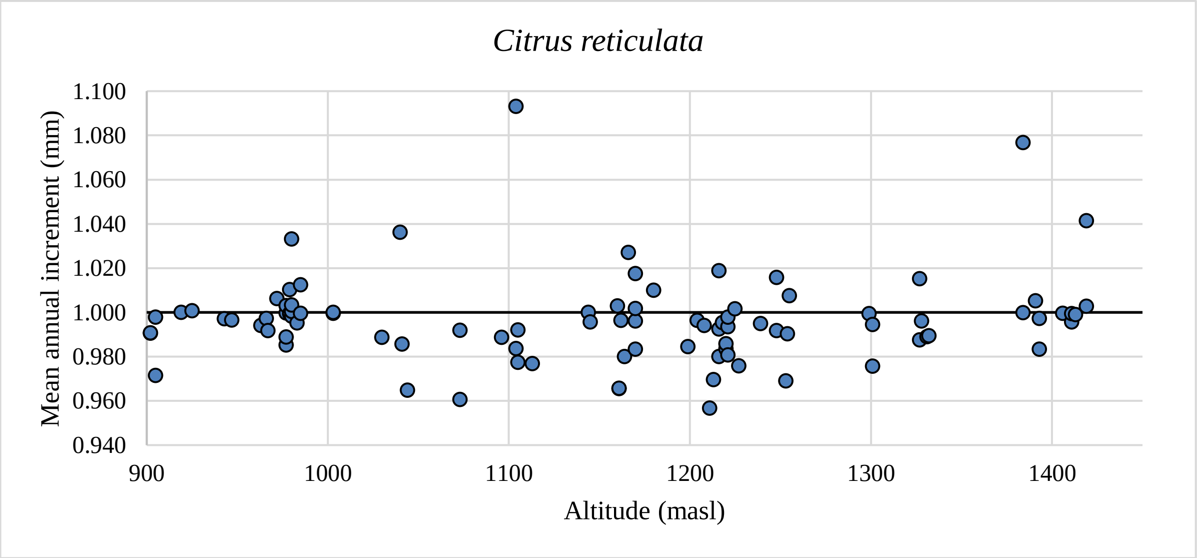

Figure 2: Scatter plot for growth performance of *C. reticulata* in relation to altitude

A similar finding was also reported by the district agriculture office of Dagana that the suitable altitude for the growth of *C. reticulata* is normally 1,100 masl. However, in recent years, radial growth has shifted to 1,200 masl (Delma, 2018), based on fruit production in upper and lower regions. According to RNRRC

(2008), the greatest elevations at which *C. reticulata* are currently growing are between 300 and 1,500 masl. The study by Joshi and Gurung (2009) also revealed a progressive increase in mean annual increment towards the altitudinal range of 1,300 and 1,450 masl. It validates that the growth of *C. reticulata* is shifting within these ranges. However, the growth trend continues to show a gradual increment.

As per Balfagon, Arbona, and Gomez-Cadenas (2021), in the areas where citrus types are now being cultivated, the environment is changing due to the rise in temperature as well as other unfavorable climatic phenomena. With rising temperatures, it is anticipated that the detrimental effects of these unfavorable weather conditions such as dryness or higher soil salinity on citrus physiology and production would worsen, threatening the crop productivity and, in extreme cases, survivability of the plant (Shrivastava and Kumar, 2014). By investigating the responses of citrus tolerance to climate change, it might be feasible to develop new varieties of citrus that can withstand shifting climatic conditions while still producing.

Radial growth performance of P. roxburghii on altitude levels

When the mean annual increment of *P. roxburghii* was compared across altitude levels, the results (Table 4) indicated that altitude had no significant effect on the species' growth *(F* (1, 103) = 2.525*, p* = 0.115*)*. However, the graphical representation indicated that the altitudinal range of 1,000 to 1,150 masl had the optimally higher mean annual increment. The highest mean annual increment (1.033 mm) was also observed at an altitude of 1,090 masl (Figure 3). This is probably because the majority of the trees within that altitudinal range were young (40 to 60 years) (Figure 4). The study also revealed a substantial mean annual increment of *P. roxburghii* within the altitudinal range of 1,150 to 1,450 masl, however, it continued to fluctuate within the ranges of 600 to 2,300 masl in Bhutan.

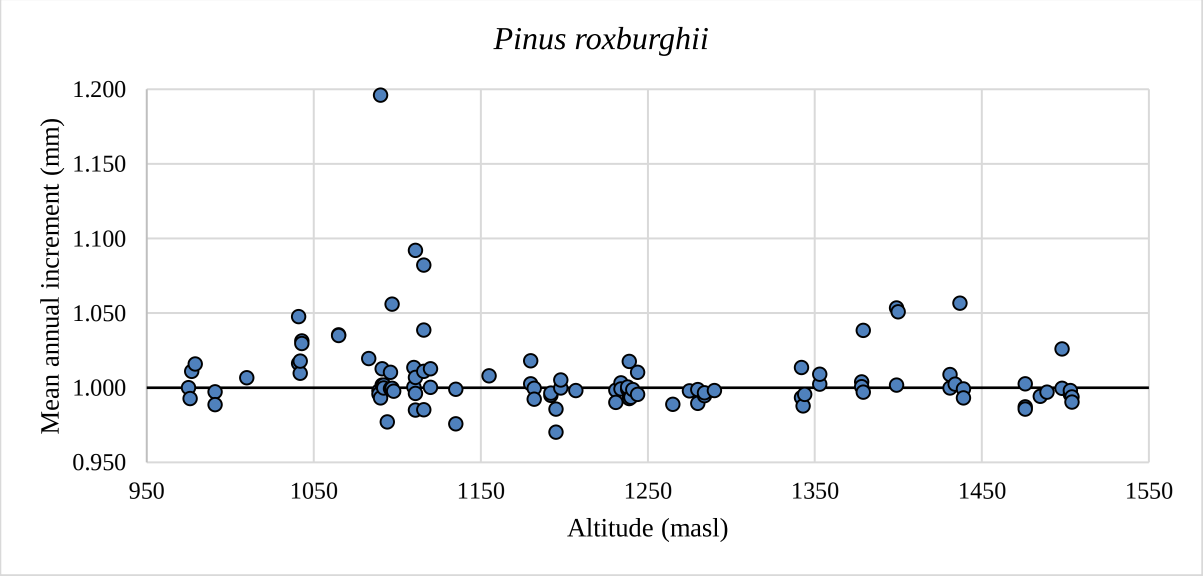

Figure 3: Scatter plot for growth performance of *P. roxburghii* in relation to altitude

Table 4: Radial growth performance of *P. roxburghii* by altitude

Sl.no.		*SS*	*df*	*MS*	*F*	*p*
1	Between Groups	0.002	1	0.002	2.525	0.115
2	Within Groups	0.076	103	0.001		
3	**Total**	**0.077**	**104**			

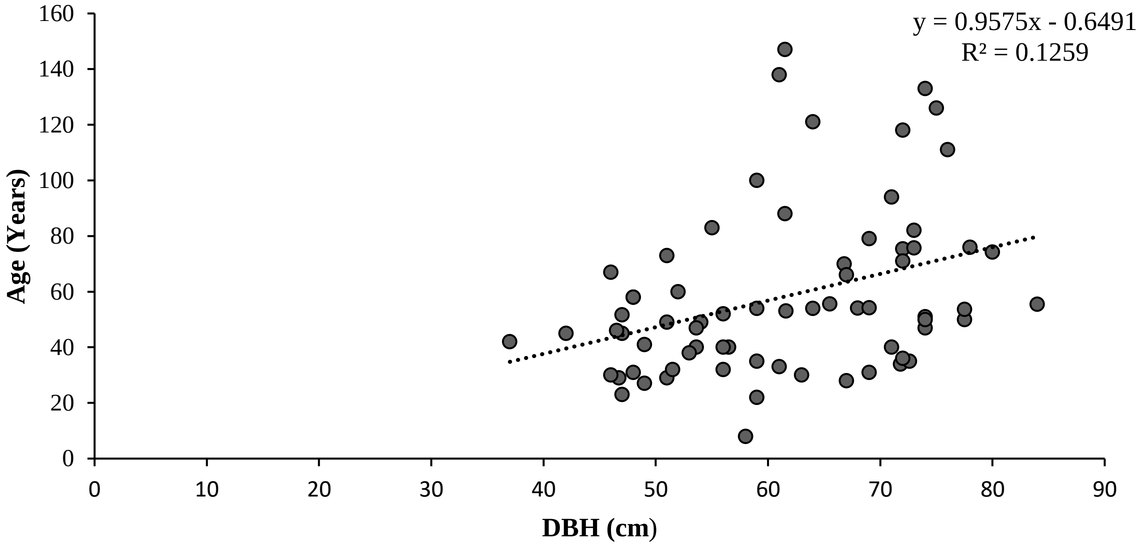

Figure 4: Scatter plot for age in relation to DBH in *P. roxburghii*

Analysis of C. reticulata growth variance

To compare the growth performance of an upper and lower site, composite tree-ring chronologies have been developed for both the species from the upper site and lower site. Neither of the chronologies sustained persistent growth suppression to unpredictable change in periodicity. Both composite chronologies showed considerable annual variation in the growth of C. *reticulata.* The radial growth was low in the 1990s and 2010s in both the regions and in the 1980s in lower regions. The highest observed growth was between the years 2005 to 2008. The temporal variations of annual citrus growth are more prominent in the lower region. General correspondence of citrus ring-growth variations ($r = 0.458$, $p < 0.05$) for the common period 1986 to 2021) suggests that one or more common factors limit citrus growth across the studied region span, also affecting tree growth.

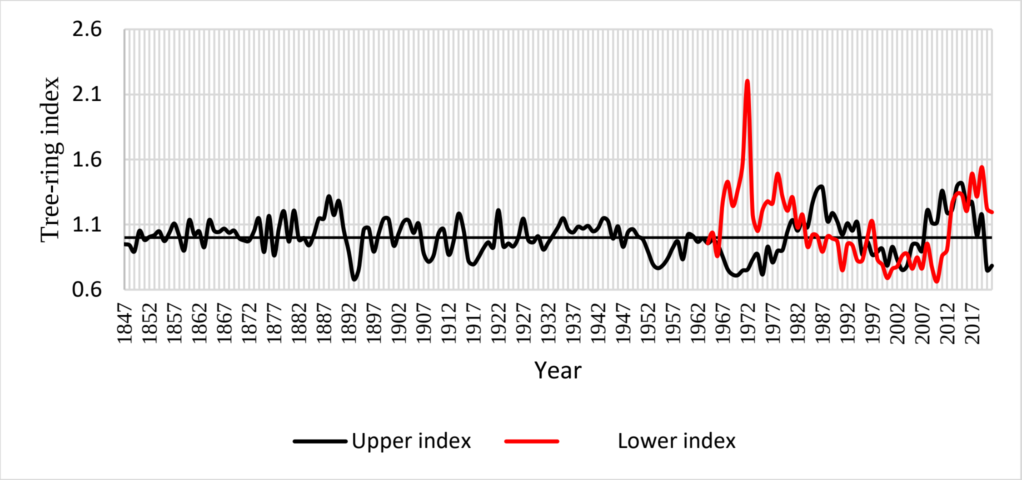

Figure 5: Composite tree-ring chronologies of *C. reticulata* from the upper site (black) and lower site (red). The chronologies correlate significantly for the common interval 1986 to 2021, $r = 0.458$, $p < 0.05$.

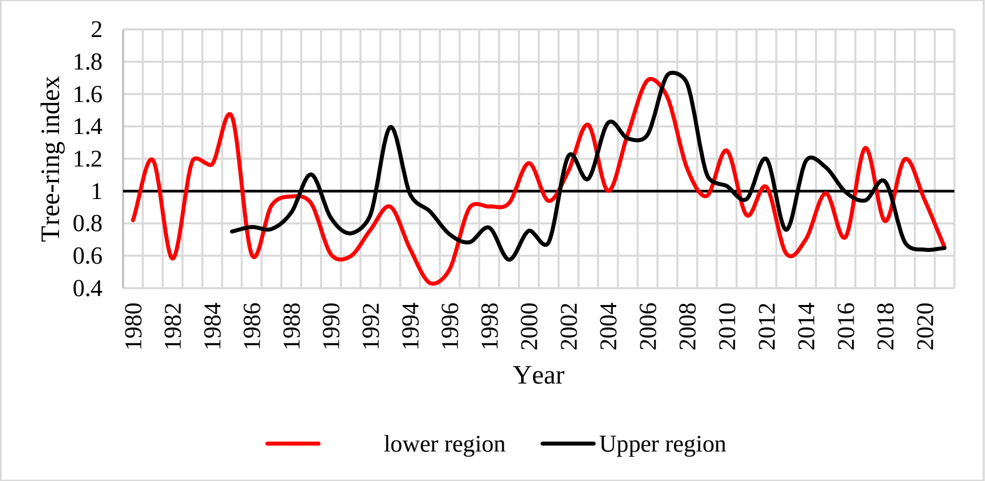

Figure 6: Composite tree-ring chronologies of *P. roxburghii* from the upper site (black) and lower site (red). The chronologies correlate significantly for the common interval of 1964 to 2021 ($r = 0.223$, $p < 0 .05$).

Both the composite chronologies showed considerable annual variation in the growth of *P. roxburghii*. The radial growth was low in the 1980s, 1990s, and 2000s in both the regions, and in the 1890s, 1950s, 1960s and 1970s in the upper region. The highest observed growth was between the years 2009 to 2021 in both the regions, between 1964 to 1985 and 1982 to 1993 in the lower and upper regions, respectively. The temporal variations of annual *P. roxburghii* growth are more prominent in the lower region. Similarly, the general correspondence of chir pine ring-growth variations ($r = 0.223$, $p < 0.05$ for the common period of 1964 to 2021) suggests that one or more common factors limit *P. roxburghii* growth across the studied span of the region.

Influence of growth factors in the upper study site

Multiple regression analysis, with the dependent variable as log value of mean increment, was used to determine if radial growth of *C. reticulata* and *P. roxburghii* can be influenced by other growth factors (independent variables in numeric scale) other than climate. It was expected that altitude, slope percentage, and the basal area will positively predict radial growth. For the *C. reticulata* and *P. roxburghii* of the upper study site, collectively, results show that variance in radial growth can be poorly accounted for by the three predictors, F (3, 48) = 1.251, p>0.001 and F (3,70) = 1.645, p>0.001, respectively. The individual contributions of the predictors for *C. reticulata* (Table 5) and *P. roxburghii* (Table 6) showed not a single individual predictor having significant influence.

Table 5: Individual contributions of the predictors for *C. reticulata*

Sl. no	***Model***	***B***	***SE***	***β***	***t***	***p***
1	(Intercept)	0.944	0.04	0.206	23.674	0
2	Altitude	0.042	0	0.17	1.471	0.148
3	Slope (%)	0	0	-0.159	1.154	0.254
4	Basal area (m$^{2)}$	-0.308	0.283	0.206	-1.09	0.281

$R^{2=}$ -0.054, p>0.001

Table 6: Individual contributions of the predictors for *P. roxburghii*

Sl. no.	***Model***	***B***	***SE***	***β***	***t***	***p***
1	(Intercept)	1.008	0.034		29.549	0
2	Altitude	-0.01	0	-0.054	-0.459	0.648
3	Slope (%)	-0.04	0.033	-0.151	-1.226	0.224
4	Basal area (m$^{2)}$	0	0	0.242	1.955	0.055

$R^{2=}$ 0.026, p>0.001

Influence of growth factors in the lower study site

For the *C. reticulata* and *P. roxburghii* in the lower study site, collective result showed that variance in radial growth cannot be significantly accounted for by the three predictors, F (3,37) = 0.314, p>0.001 and F (3,27) = 0.204, p>0.001, respectively. The individual contributions of the predictors for *C. reticulata* (Table 7) and *P. roxburghii* (Table 8) showed not a single individual predictor having significant influence. This study depicted that there were no significant influences on the mean annual increment of both the species by the growth factors validating the minimal existence of signal-to-noise ratio indicating the fitness of tree-ring data for climatic influence analysis.

Table 7: Individual contributions of the predictors for *C. reticulata*

Sl. no	*Model*	*B*	*SE*	*ß*	*t*	*p*
1	(Intercept)	1.014	0.023		43.487	0
2	Altitude	-0.015	0	-0.119	-0.734	0.467
3	Slope (%)	0	0	-0.092	-0.563	0.577
4	Basal area (m$^{2)}$	0.064	0.171	0.061	0.376	0.709

$R^{2=}$ -0.054, p>0.001

Table 8: Individual contributions of the predictors for *P. roxburghii*

Sl. no	*Model*	*B*	*SE*	*ß*	*t*	*p*
1	(Intercept)	0.972	0.108		8.99	0
2	Altitude	0.031	0	0.068	0.333	0.742
3	Slope (%)	0.029	0.042	0.13	0.681	0.502
4	Basal area (m$^{2)}$	-0.02	0	-0.009	-0.044	0.965

$R^{2=}$ -0.086, p>0.001

Correlation analysis between temperature and radial growth of C. reticulata

The correlation analysis between temperature and radial growth depicted that there was no significant relationship between upper radial growth index (r = -0.165, p = 0.392) and lower radial growth index (r = 0.101, p = 0.602), respectively. However, the graphical representation shows a general agreement on the pattern of rising and falling temperatures (Figure 7). In both the regions, the highest radial growth index for *C. reticulata* was recorded at the temperature range of 19 to 19.4°C between the years 2001 to 2010. The lowest was recorded at 19.5 to 20.3°C between the years 1997 to 2001 for the upper region and at 18.3 to 19.5°C between the years 1990-1997 for the lower region. The meteorological records also showed the mean annual temperature was lowest in the year 1991 (18.27°C) (NCHM, 1990-2020).

The optimum growth was found to be within the mean annual temperature of 19 to 19.4°C. Abobatta (2021) also reported similar results, indicating that citrus often grows in the range of 10 to 35°C, supporting the growth of *C. reticulata* in this ideal range. However, since 2010, the mean annual temperature has fluctuated, which has led to fluctuations in the radial growth index. Chhogyel and Hasan (2020) acknowledged this result and noted that farmers in Bhutan have suffered more losses in citrus production over the past 10 years, and they were cautioned that the mean annual temperature would continue to fluctuate in the near future.

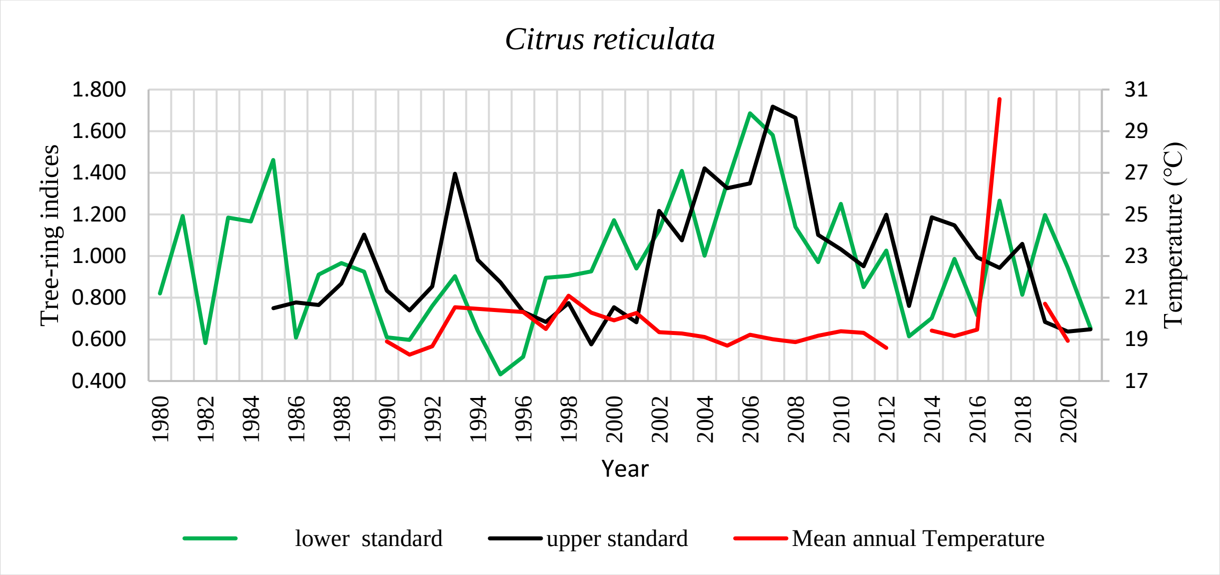

Figure 7: *C. reticulata* growth trend in relation to the mean annual temperature (1990 to 2020)

Correlation analysis between temperature and radial growth of P. roxburghii

Similarly, correlation analysis between the radial growth index of *P. roxburghii* of both upper and lower regions with the mean annual temperature depicted that there was no significant relationship between them, $r = 0.175$, $p = 0.363$ and $r = 0.438$, $p = 0.018$, respectively. However, from the graphical representation, there is an interesting feature, which may be an art of processing method; the rate of radial growth of both the region appears to precede the increase in temperature (Figure 8).

The optimal radial growth index of *P. roxburghii* was observed at the mean annual temperature range of 18.70 to 30.54°C between the years 2007 to 2020 and the lowest at 18.8 to 19.91°C in both the regions between 1997 to 2004. Both the highest and lowest radial growth index of *P. roxburghii* in relation to annual mean temperature was observed in recent years relative to *C. reticulata*. This was reported to be due to *C. reticulata* having slow growth rate and being more complex compared to *P. roxburghii*, which tends to show relatively low climatic sensitivity (Silvy et al., 2018). The graphic patterns of growth revealed a high radial growth index when the mean annual temperature was low and the radial growth index anticipated the subsequent rise in mean annual temperature is due to climate change (Chauhan et al., 2017).

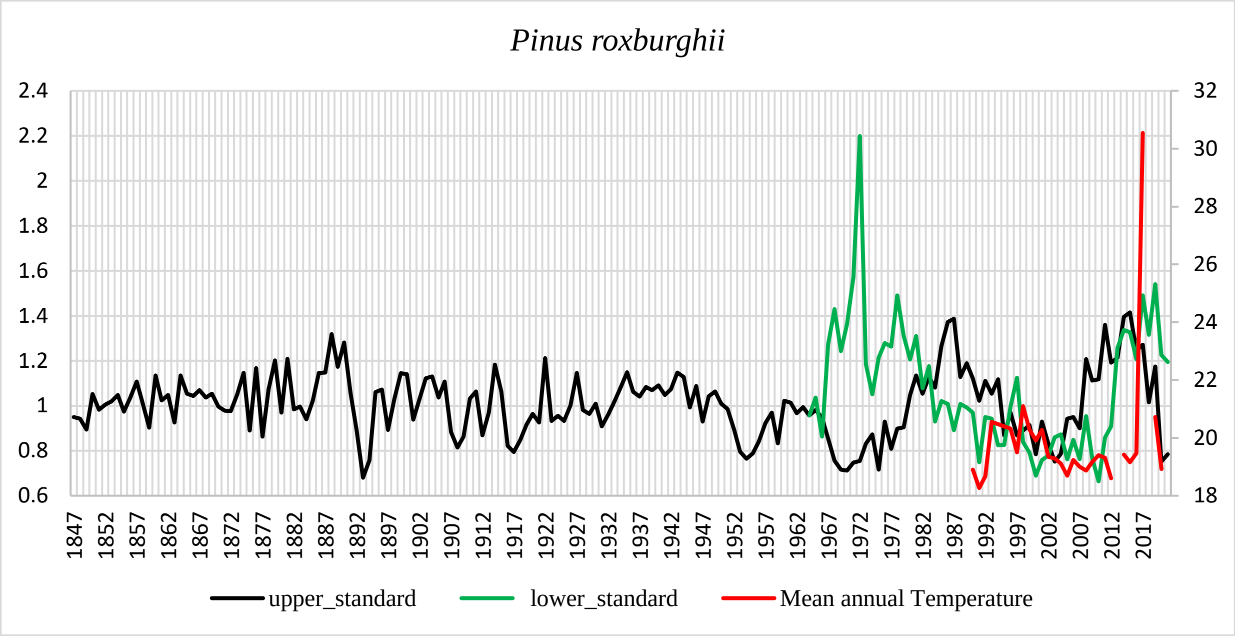

Figure 8: *P. roxburghii* growth trend in relation to the mean annual temperature (1990 to 2020)

Correlation analysis between precipitation and radial growth of C. reticulata

The correlational analysis between the mean annual precipitation and the radial growth index of the upper and lower regions did not significantly correlate (r = -0.359, p = 0.056 and r = -0.340, p = 0.071, respectively). However, general graphical agreement of rising and falling precipitation tends to depict a negative correlation at some point in time (Figure 9). According to Boakye et al. (2021), hardwoods are believed to depict a positive relation with rising mean annual precipitation which in this study could have been affected by other limiting factors.

In both regions, the optimal radial growth index was observed at the mean annual precipitation range of 86.51 to 147.45 mm between the years 2004 to 2010 when the mean annual precipitation was relatively low. The lowest was observed at 95.25 to 216.72 mm between the years 1995 to 2001 when the mean annual precipitation was relatively higher. Due to their larger vessels that allows for higher hydraulic conductance, higher mean annual precipitation is expected to favor *C. reticulata* growth. However, according to field observations in this study, lower growth with rising mean annual precipitation may be associated with periodic insect outbreaks that impact the tree growth trajectories (Boakye et al., 2021).

According to Joshi and Gurung (2009), the majority of the currently growing citrus is found within the average annual rainfall of 1,000 mm, but in this study, it was found to be relatively low average annual rainfall for maximum growth. This might have happened due to the changing pattern of rainfall by climate change, considerably decreasing rainfall in subtropical regions from 2005 to 2014 (Tenzin et al., 2019).

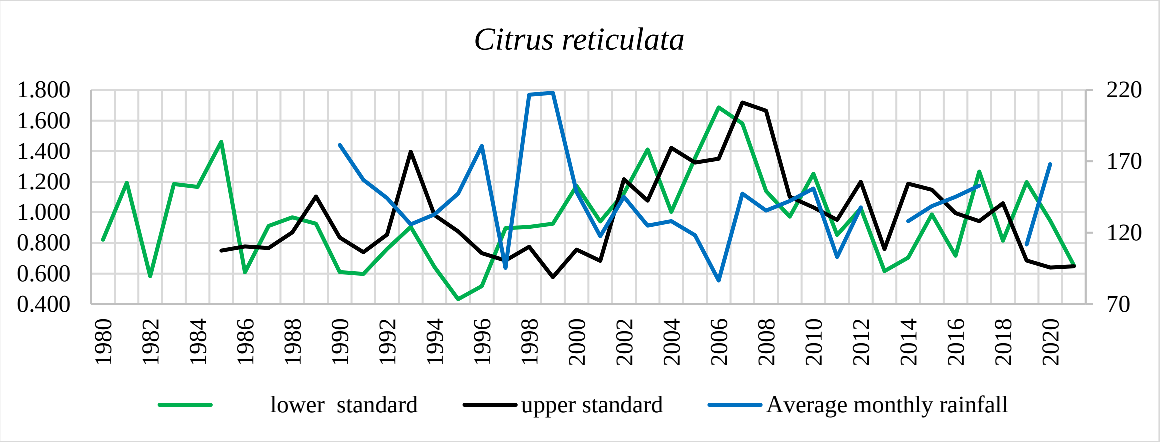

Figure 9: *C. reticulata* growth trend in relation to average monthly precipitation (1990 to 2020)

Correlation analysis between precipitation and radial growth of P. roxburghii

Correlation analysis between the radial growth index of *P. roxburghii* in both upper and lower study regions and mean annual precipitation showed no significant relationship between them, $r = -0.164$, $p = 0.395$ and $r = -0.119$, $p = 0.539$, respectively. This can be due to *P. roxburghii* being a light-demanding species, and fluctuating light intensity during monsoon season could have possibly been the affecting factor behind the insignificant correlation between radial growth index and precipitation (Nautiyal et al., 2019). The graphical representation shows that the rate of growth of the radial growth index precedes the increasing mean annual precipitation, which may be due to the effect of previous precipitation that could not be assured due to the unavailability of long instrumental precipitation records (Figure 10).

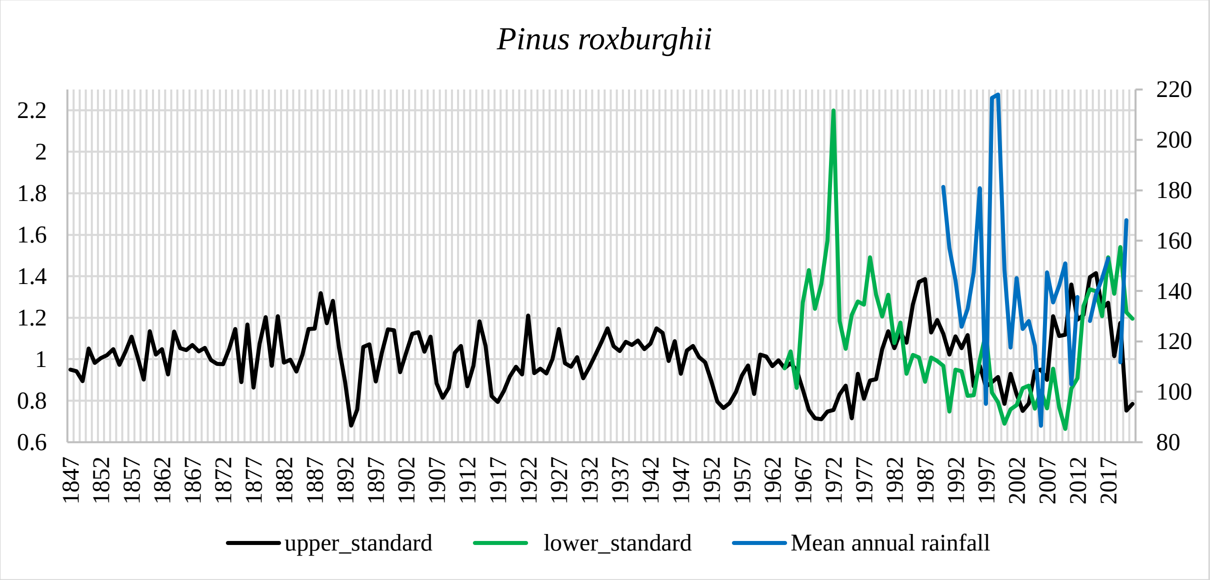

Figure 10: *P. roxburghii* growth trend in-relation to mean annual precipitation (1990-2020)

In upper study site, the ideal growth mean index of *P. roxburghii* was found to be between 103 and 168 mm between 2011 and 2020, while the lowest was found to be between 86.51 and 95.25 mm between

1997 and 2006 in both regions. The relatively better performance of *P. roxburghii* in relation to fluctuating mean annual precipitation can be related to its resistance to seasonal water deficits than *C. reticulata* due to their earlier narrower tracheids (Boakye et al., 2021)**.**

Conclusion and recommendations

Despite being separated by an altitudinal range of 400 to 450 masl, the two study sites in Dagana show no appreciably different growth variability. The strongest radial growth of C. reticulata was observed in the altitudinal range of 1,150 to 1,250 masl. The correlational analysis of tree-ring growth performance with the meteorological data showed that the growth performance of *C. reticulata* was highest at a mean annual temperature range of 19 to 19.4°C and a mean precipitation range of 86.5 to 147.45 mm. In terms of mean annual temperature and precipitation, *P. roxburghii* tends to have a negative relationship whereas *C. reticulata* tends to exhibit a positive association. However, the growth variability of both *C. reticulata* and *P. roxburghii* in both regions depicted unprecedented variability from the fluctuating climatic factors.

The results obtained from this research can contribute as impact estimation material for the impacts of changing climate and in addition, based on the statistical analysis between the tree-ring growth and climatic factors, it can be useful in estimating the change in productivity due to changing climate in the future. Furthermore, the shift in the habitat of *C. reticulata* was observed but could not determine the rate of shifting therefore, demarking the upper and lower altitudinal limit for the *C. reticulata* and *P. roxburghii* will assist in studying shift and effect of climate change in species distribution. The detailed studies on the relationships between climatic change and dynamics incorporating multiple species, covering other proxy evidence are needed.

Conflict of Interest

None.

Authors' Contribution Statement

Dorji Nidup conceived the general idea, collected and analyzed data, and wrote and edited the content. Yeshey Khandu edited contents and guided throughout the process. Tashi Dendup, Jigme Chozang, and Ugyen Wangmo contributed ideas, insights, and suggestions for better results and edited the contents.

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