INTERNATIONAL JOURNAL OF ENVIRONMENT

Volume-11, Issue-2, 2022 ISSN 2091-2854

Received: 16 March 2022 *Revised: 14 July 2022* *Accepted: 19 July 2022*

ECOLOGY OF PERSIAN WALNUT (*Juglans regia* L.) IN WESTERN BHUTAN

Laxmi Sagar[1,*], Jambay[2], Ngawang Gyeltshen[3], Bhakta Bdr. Ghalley[3], Sonam Yonten[3], Namkha Gyeltshen[1], Rupesh Subedi[1] and Rinchen Dorji[1]

[1]College of Natural Resources, Royal University of Bhutan

[2]Department of Forest Science, College of Natural Resources, Royal University of Bhutan

[3]Department of Forest and Park Services, Ministry of Agriculture and Forest, Bhutan

*Corresponding author: sagarlssagar19@gmail.com

Abstract

In Bhutan the Persian walnut (*Juglans regia* L.) is highly valued for its timber and other medicinal properties and is listed under special class in Royalty on Forest Products of Bhutan. However, the ecology of the species in the country is poorly understood. Therefore, this study aimed to understand the ecological requirements and habitat modeling of the species in Bhutan. A total of 20 plots (five plots in each district) were laid using purposive non-probability sampling in four districts (Gasa, Punakha, Wangdue Phodrang, and Dagana) of Western Bhutan. Plot size of 20 m X 20 m (major plot) for tree and understory and 2 m X 2 m (minor plot, nested within the major plot) for ground cover were used to collect vegetation data. Soil samples (N = 20) were collected from the center of the major plot from a depth of 25–30 cm. A total of 163 plant species belonging to 74 families were recorded. Pearson and Kendal correlation of CCA Ordination showed moderate influence of slope (r = 0.66) followed by altitude (r = 0.55). Annual mean temperature and rainfall showed moderately negative correlation with the growth and distribution of *J. regia* (r = -0.54 and -0.64) in the study area. Suitable habitat was obtained using fuzzy overlay in ArcGIS. Overall, 18.61% of the country's area was found to be suitable for growth and development of the species. This area can be used for the protection and management of the species in future.

Keywords: Ecology, habitat, Persian walnut, vegetation, western Bhutan

DOI: https://doi.org/10.3126/ije.v11i2.43844

Introduction

Juglans regia L. (Persian Walnut) is an economically important plant species (Querné et al., 2017) belonging to genus Juglans under the family Juglandaceae (Manos and Stone, 2001). According to Dong et al. (2017), genus Juglans has an estimated 21 different species, of which majority of them not only have edible and nutritious nuts, but also produce high quality, durable, and decay -resistant timber, which is highly valued in the global market (Chen et al., 2019). From the global estimate of 21 species, *J. regia* and *J. nigra* are considered to be the most common species which are widely distributed and commercially cultivated (Burtin et al., 1998). *Juglans regia* is the oldest cultivated species in the history of mankind for timber and nuts (Thakur, 2011) and is found in its natural state in central and other Asian countries (Molnar, 2011), including Bhutan (Zohary et al., 2012).

Juglans regia is regarded as one of the best commercial timber species (Rigo et al., 2016) but very little is known regarding the species despite holding high significance in economic landscape (Guo et al., 2020). The natural stand of *J. regia* in Bhutan has been reported from evergreen oak forests in various pockets of the country including Dagana, Chhukha, Punakha, Wangdue Phodrang, and Gasa among others (Grierson and Long, 1983; Department of Forest and Park Services [DoFPS], 2012). Jain and Priyadarshan (2009) reported that the species is usually found growing in mixed stands or as scattered individual trees but rarely as pure stand.

In Bhutan apart from natural growing stands, native inhabitants grow the species due to commercial potential of the nut in the national market (Ghimiray and Katwal, 2013). Natianal Statistics Bureau [NSB] (2021) reported about 26,061 trees grown by local people of which 8,708 are in bearing stage. In the year 2021 approximately 179 metric ton (MT) of nut was produced in the country (NSB, 2021). Mariana and Niculina (2017) stated that various factors influence the growth and development of *J. regia.* Cosmulescu et al. (2010) highlighted that ecological factors play decisive role in growth and yield of the *J. regia*. Gauthier and Jacobs (2011) emphasized light, rainfall/water, soil nutrients, and temperature as the major factors contributing to the growth and distribution of *J. regia*. However, lack of scientific studies in the country increases exploitation of the species resulting in accelerated population decline. The high timber value and medicinal use of *J. regia* has caused illegal felling thereby posing risk to population in the wild.

Though Bhutan has placed *J. regia* under special class of timber (Department of Forests [DoF] 2006), very sparse information is available in the country regarding the growth and distribution of the species. The variation in the environmental attributes in different sites is expected to contribute differently to the growth and development of the species. Therefore, this study aims to explore the vegetation composition, assess environmental attributes, and model suitable areas for growth of *J. regia* in the country. The information generated through this study is expected to inform the policy makers and practitioners to be aware of basic ecological requirements of the species in the country and help make better decision on sustainable management as well as plantation of the species for timber production.

Materials and methods

Study area

Owing to its occurrence in small pockets of the country, current study focused in four districts, namely, Dagana, Gasa, Punakha, and Wangdue Phodrang (Fig. 1), where the species is found (Grierson and Long, 1983; DoFPS, 2012). Gasa district lies in the extreme northwest of the country (RGoB, 2018b), and has an elevation ranging from 1,000 to over 7,000 masl and falls entirely under Jigme Dorji National Park (JDNP) (DoFPS, 2021). The district experiences a total annual rainfall of 2,509.3 millimeters (mm) and the average annual temperature ranges between 4.5ºC to 14.3ºC (National Centre for Hydrology and Meteorology [NHMC], 2020). About 19.06% of the district is under forest cover (Forest Resource and Management Division [FRMD], 2017). Situated in western Bhutan (RGoB, 2019), Punakha district has an elevation ranging from 1,300 masl to 4,800 masl experiencing cool winter and warm to hot summer. The average annual temperature ranges between 16.3ºC to 28.3ºC. It experiences a total annual rainfall of 553.1 mm (NHMC, 2020). Approximately 83.63% of the district is under forest cover (FRMD, 2017). Wangdue Phodrang lies in the central part of the country and occupies an area of approximately 4,035.65 km^2 (FRMD, 2017).The district has an elevation ranging from 500 masl to 7,070 masl. The annual average temperature ranges between 15.6ºC to 25.1ºC and experiences a total annual rainfall of 643 mm (NHMC, 2020). Dagana district is located between the deep valleys of Thimphu and Wangdue Phodrang, and lies in the southwest region of Bhutan (RGoB, 2018a) with elevation ranging between 750 masl to 2,000 masl, and experiences cool winter and hot summer (FRMD, 2017). The annual temperature ranges from 13.2ºC to 22.4ºC with a total annual rainfall of 1,316.7 mm (NHMC, 2020). Almost 89.14% of the district is under forest cover (FRMD, 2017).

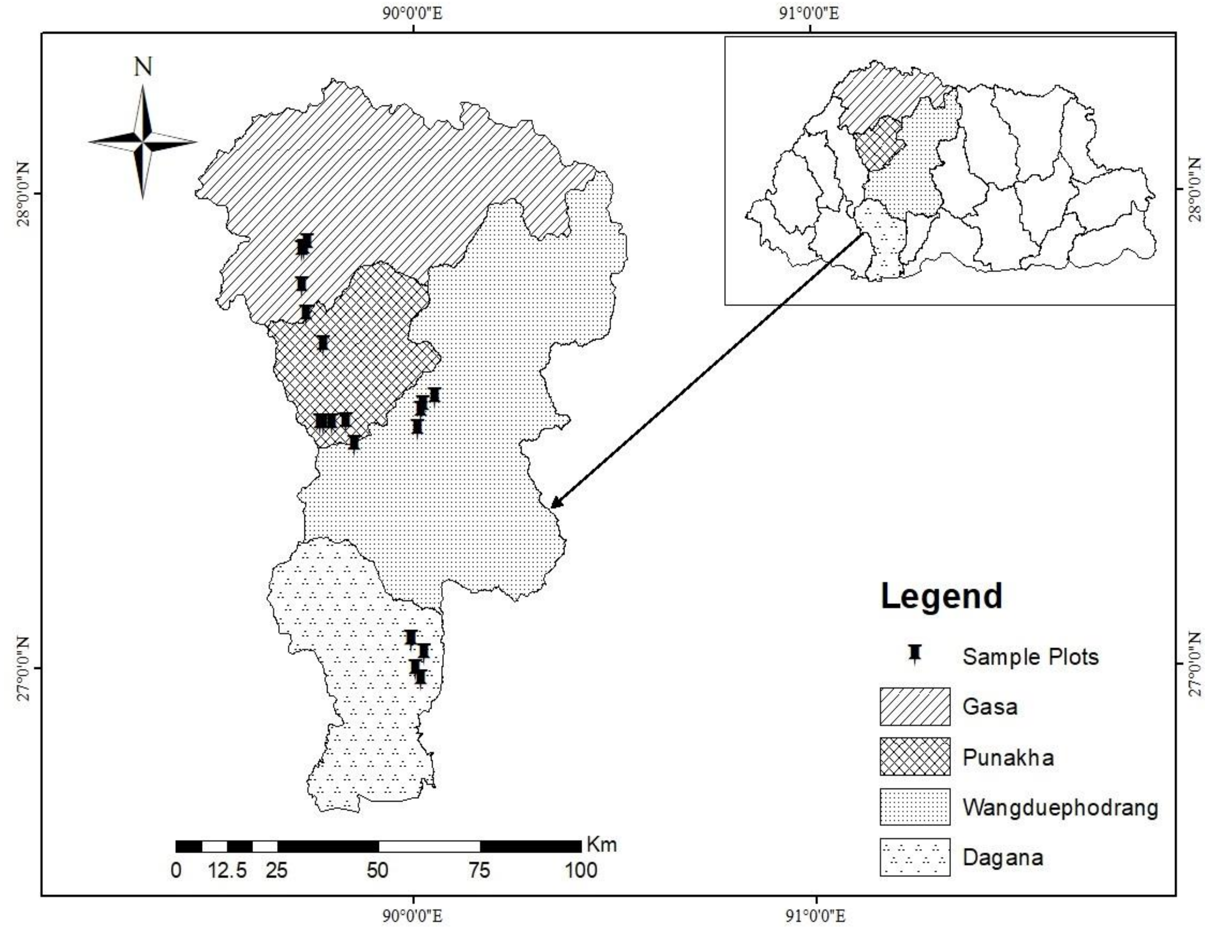


Fig. 1. Location map of study area showing four districts and the sample plots

Plot layout and vegetation survey

Juglans regia is sparsely distributed in the country, so a non-probability purposive sampling was used to collect the primary ecological attributes, namely, climatic, edaphic, biotic, and topographic, influencing the growth and distribution of the species (DoFPS, 2012; Acharya et.al 2013; Rabgay et al., 2020) For a univariate (single) population the optimum sample size and plot area depend upon the spatial distribution of the target species and the resource and time available (Kenkel and Podani, 1991). A total of 20 plots in four different districts were established (five plots in each district) based on available resources and local information.

The study adopted plot design following DoFPS (2020) where a major plot size of 400 m^2 (20 m X 20 m) was established in each site for tree and shrub. Within the major plot a minor plot of 4 m^2 (2 m X 2 m) was nested for collection of herb data (DoFPS, 2020). Soil samples were collected from a depth of 25–30 cm from the center of the major plot (Clay et al., 2002).

From the major plot, all tree species with diameter ≥ 10 cm at Diameter at Breast Height (DBH) and height of the species were recorded (Kaka et al., 2014). In addition to tree species, all shrub species were also recorded by taking individual count of each species (DoFPS, 2020). All herbaceous plants were enumerated from the minor plot.

Climatic and topographic data collection

Environmental variables such as altitude, slope, aspect, and location having significance influence on the growth and distribution of *J. regia* were collected using an altimeter, compass, clinometer, and Global Positioning System (GPS), respectively. For the collection of climatic variables such as rainfall and temperature effecting the growth of the species, the models developed by Dorji et al. (2016) were used.

Vegetation data analysis

Vegetation data collected from the field was cleaned and sorted using Microsoft Excel followed by the computation of species basal area (BA) from the DBH data of individual trees with DBH ≥ 10 cm and height ≥ 130 cm (Kaka et al., 2014). Similarly, relative Basal Area (RBA) in percent was computed for each tree species. Relative Basal Area was used to determine the vegetation structure present in *J. regia* stand. The DBH data was used to analyze stand structure and distribution pattern of *J. regia* forest (Wangda and Ohsawa, 2006a). To explain the floristic composition of shrub and herb, density and relative density (RD) were established using count of individual species.

Soil data analysis

The soil samples were analyzed in Soil, Water, and Air Testing (SWAT) laboratory at the College of Natural Resources (CNR), Royal University of Bhutan (RUB). The variables included were soil pH, nitrogen (N), phosphorus (P), and potassium (K), soil organic carbon (OC), moisture content (MC), and soil organic matter (SOM).

Soil pH was analyzed using automatic pH meter (Pakale et al., 2018). Soil N was determined using Kjeldhal method (Bremner, 1960), Soil P using Bray II extraction method (Bray and Kurtz, 1945) and soil K using flame photometric method (Knudsen et al., 1982) after processing the soil samples using multiple extraction procedures (Bhattarai and Tomar, 2009). Soil OC and SOM was determined using Loss on Ignition method (Dean, 1974).

Habitat modeling

Suitable habitats for the growth of *J. regia* in Bhutan was analyzed using fuzzy overlay in ArcGIS. Both vector and raster variables were used for the modeling. Vector data such as land use land cover, river, road, and forest types were obtained using Land Use and Land Cover of Bhutan (LULC) 2020. Raster data such as slope, aspect, elevation, temperature, and precipitation were extracted from Digital Elevation Model (DEM) using spatial analyst tool in ArcMap (Rabgay et al., 2020). A proximity buffer of 100 m and 50 m was maintained for roads and rivers respectively.

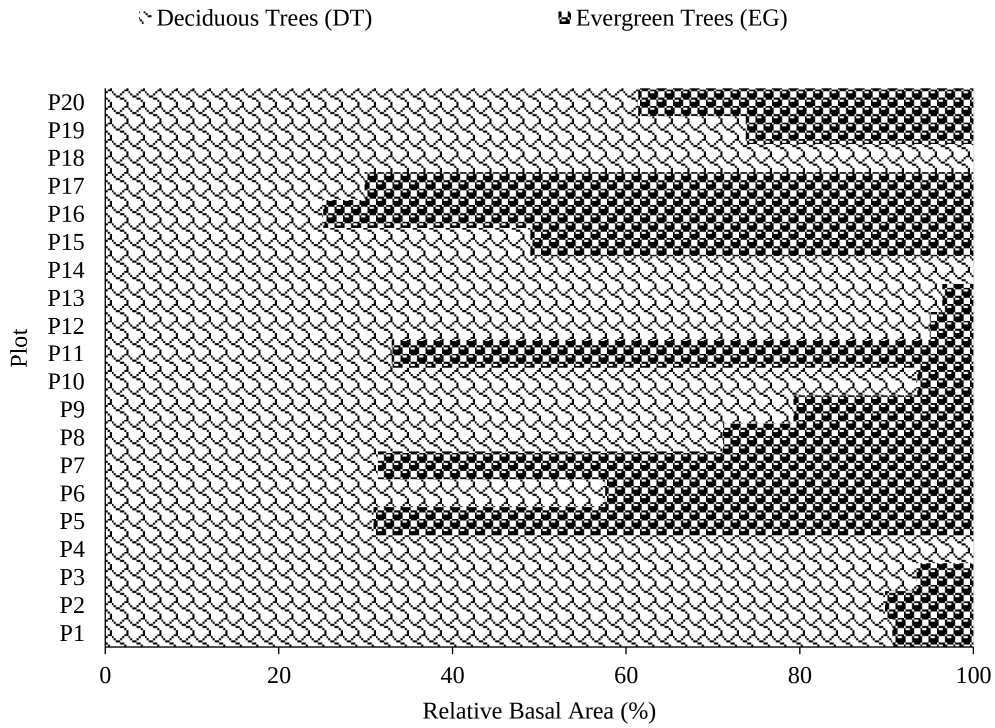
Statistical analysis

All the data processed in Microsoft Excel were analyzed by using PC_ORD software v5.1. Cluster analysis was performed using distance measure of Relative Sorensen's (Bay-Curtis) method and Group average as linkage method to determine the forest types. To determine the relationship between *J. regia* and environmental variable, Canonical Correspondence Analysis (CCA) ordination was performed on the forest structure of *J. regia* using RBA from each plot as the main matrix data and environmental variables as the second matrix data (Wangda and Ohsawa, 2006a). Pearson and Kendall correlation (*r*) was used to determine the strength of correlation of variables between axes (Wangda and Ohsawa, 2006a).

Results and discussion

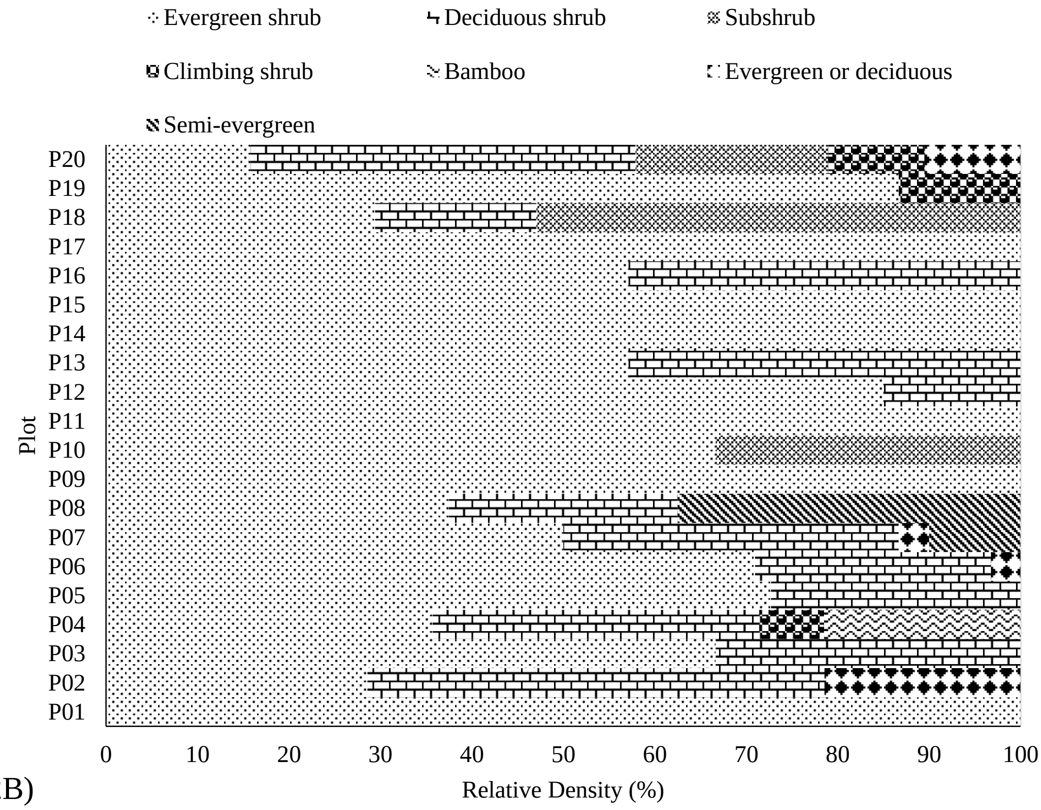
J. regia vegetation composition

A total of 163 plant species belonging to 74 families were recorded from the 20 plots. The floristic vegetation was classified under three major growth forms; tree, understory, and ground cover.



(2A)

Major lifeform trees had a total of 49 tree species belonging to 26 families. The most dominating lifeform was evergreen trees (57.14%) followed by deciduous trees (42.86%). Majority of the plots (*n* = 17) had evergreen and deciduous trees except three plots (P4, P14, and P18) which were completely dominated by deciduous trees (*RBA = 100%)* (Fig. 2A).



(2B)

The understory vegetation consisted of 49 species belonging to 27 families. The evergreen shrub constituted maximum proportion of understory vegetation (59.18%) followed by deciduous shrubs (22.45%) and bamboo and semi-evergreen shrubs the least (2.04%) (Fig. 2B). The top three most dominating species in decreasing order were Thymelaeaceae (*Daphne bhoula* Buch. -Ham. ex D. Don), Berberidaceae (*Berberis asiatica* Griff.), and Rosaceae (*Rosa microphylla* Roxb*).*

The groundcover vegetation consisted of 66 species belonging to 35 families. Perennial herb dominated the groundcover vegetation (56.06%) followed by fern (16.67%) while moss, orchid, and biennial herb were least dominating (1.51%). Rosaceae (*Fragaria nubicola* Lindl.), Urticaceae (*Girardinia diversifolia* (Link) Friis), and Urticaceae (*Pilea* sp.) were in abundance in *J. regia* vegetation.

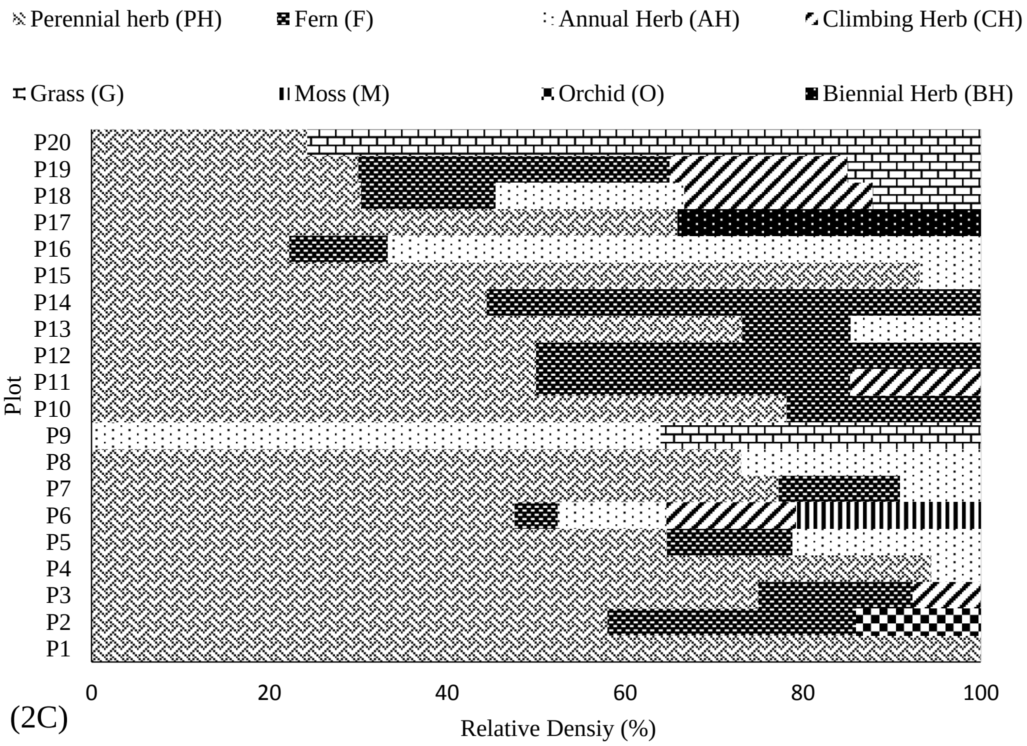


Fig. 2. *J. regia* vegetation composition, (2A): Distribution of major lifeform tree in *Juglans regia* habitat, (2B): Distribution of major lifeform shrub in *Juglans regia* habitat, (2C): Distribution of major lifeform groundcover in *Juglans regia* habitat

Stand structure and distribution pattern of Juglans regia

Three stand distribution types, namely, *Inverse J* (showing excellent regeneration pattern/ status), *Sporadic* (showing good regeneration pattern/ status), and *J-Shaped* (indicating poor recruitment of tree species) were observed (Teketay, 2005) (Fig. 3).

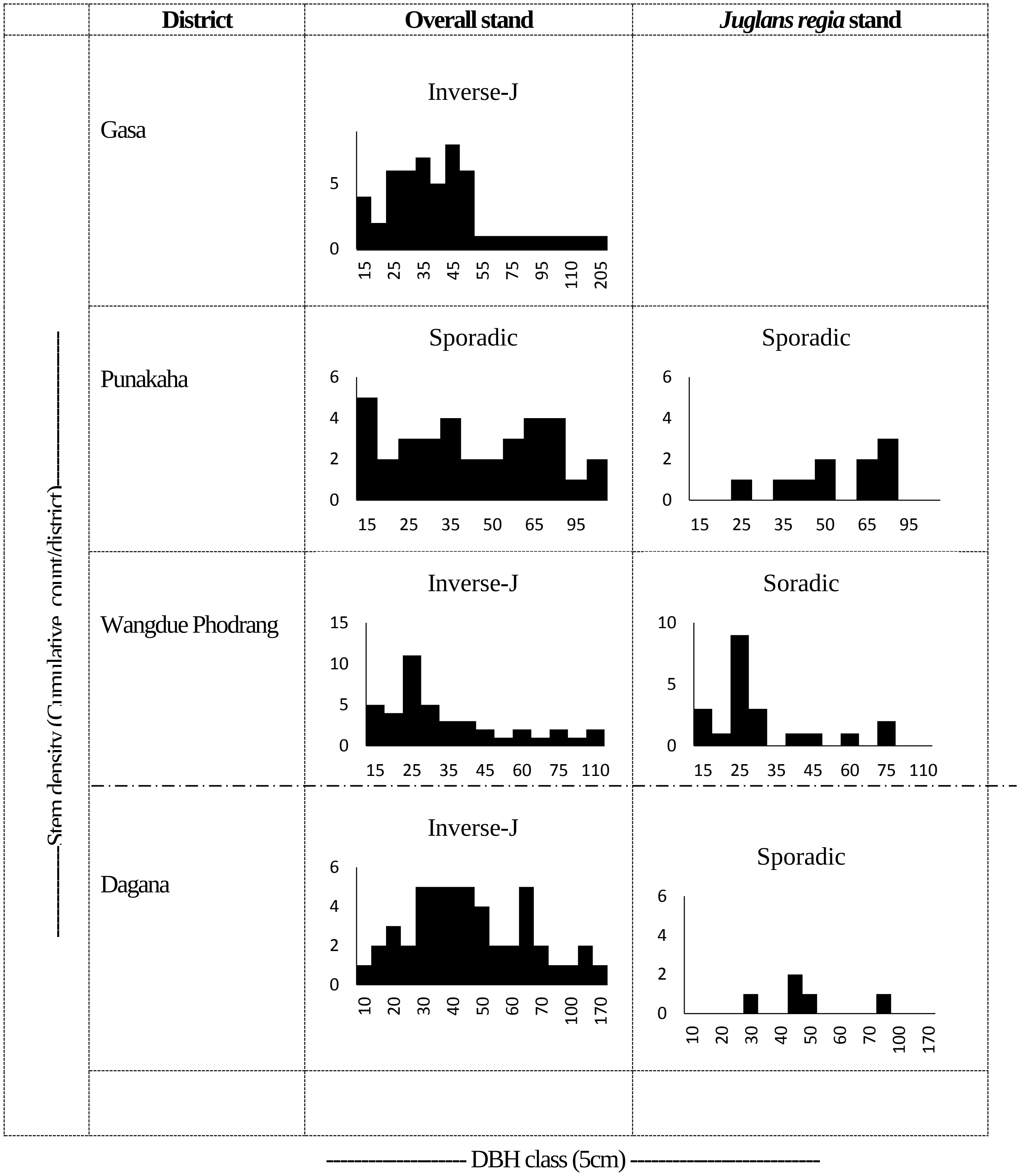


Fig. 3. Stand structure and distribution pattern of overall tree stand and *J. regia* stand in the study area

The overall stand distribution pattern in *J. regia* habitat was performing well, portraying an *inverse-J* distribution in three districts (Gasa, Wangdue Phodrang, and Dagana). However, Punakha district displayed *sporadic* distribution showing good regeneration status but with human intervention (Tesfaye et al., 2010). The distribution pattern of *J. regia* stand showed that three districts (Punakha, Wangdue Phodrang, and Gasa) had *sporadic* distribution while the remaining district (Gasa) had an *Inverse-J* which signifies unstable distribution of *J. regia* population in the study area thereby limiting continuous regeneration of the species (Rao et al., 1990; Teketay, 2005). High utilitarian value of *J. regia* has triggered mass felling of the species causing its natural population to decline along the silk road which extends from Europe to Southeast Asia including India, Nepal, Bangladesh, Bhutan until it reaches China (Vahdati, 2014; Rigo et al., 2016; Wilson et al., 2019). Additionally, illegal harvesting might have resulted in lack of mother trees in the wild thus extremely affecting natural regeneration of *J. regia*.

Environmental variable assessment

During the study *J. regia* was found at an altitude range of 1,434 masl–2,597 masl (*SD* ± 359.66*)* showing great variation in altitude, thereby enabling the species to grow. According to Arab et al. (2019), *J. regia* is found growing well at the altitude range of 1215 masl – 2823 masl. Similarly, the soil pH ranged from 5.45–6.94 with mean pH of 6.09 (*SD ± 0.41)*. For proper growth and development of *J. regia* the optimum soil pH requirement is 6.0–7.0 (Dökmen et al., 2017).

Other edaphic factor such as soil N content can often provide hindrance to the growth of the species (Fernández-Moya et al., 2019). The current study found relatively low soil N content ranging between 0.16%–0.03% with an average N content of 0.09% (*SD* ± 0.04*)*. Bremner (1960) reported that any soil with N content less than 0.7% is considered to be low. Low soil N content makes root system weak and does not support regeneration (Claassen and Carey, 2013). The study reported no regeneration of the species in the entire study area which could be attributed to low soil N. However, there are little evidences to prove whether plant species' requirement for soil N is due to adaptation or acclimation (Boczulak et al., 2014). Similarly, during the study period soil moisture content ranged from 0.2%–27.88% with average moisture content of 5.96% (*SD* ± 5.96*)* indicating higher water requirement of the species and low drought stress (Vahdati et al., 2009).

Climatic factor such as rainfall plays a significant role in the development of the species (Behrooz et al., 2019). Mohni et al. (2009) reported that the species requires an optimum precipitation between 1,000 mm–1,200 mm per year and the current study area also had a similar annual precipitation range between 796.35 mm–3,170 mm. The annual temperatures ranged between 13.13^{o}C–19.05°C with a mean temperature of 16.07°C (*SD* ± 1.66*)*. Fernández-Moya et al. (2019) reported that under ideal condition the species requires not lets than 10°C of average annual temperature for at least six months during its peak growing season. *Juglans regia* can grow well in areas which experiences temperatures within 0°C to 10°C (Department of Agriculutre [DoA], 2019). There should be frost-free period during flowering time and the temperature should not exceed 38°C during summer months (DoA, 2019). Mariana and Niculina (2017) reported that elevated temperature reduced the number of days of flowering. The analysis of environment factors revealed a strong significant correlation between soil N and temperature (*r = 0.6, p = .002*). Temperature has a direct influence on the overall availability of soil N and warming increases nitrogen availability in soil by increasing microbial activity (Lukac et al., 2010). Similarly, there was a positive correlation between temperature and soil potassium ($r = 0.4$, $p < .05$), while no association of soil pH was found with altitude and temperature. However, Cheng-jun et al. (2014) reported that soil pH has a negative correlation with mean temperature and rainfall. Similarly, Jeyakumar et al. (2020) highlighted that soil pH decreases with increase in altitude. The influence of climatic and topographic factors on soil pH are observed at regional scale (Cheng-jun et al., 2014). According to Reuter et al. (2008), distribution of soil pH is highly dependent on the nature of parent material. Factors associated with variation in soil pH differs with geographical location and scale (Slessarew et al., 2016).

Table 1. Environmental attributes of the study area

Environmental attributes	N	Maximum	Minimum	Mean	± *SD*
Altitude (masl)	20	2594.00	1434.00	1906.71	359.66
Aspect	20	Northwest (NW)	North (N)		
Slope (°)	20	45.00	8.00	26.85	11.36
Soil pH	20	6.94	5.45	6.09	0.41
Soil Moisture Content (%)	20	27.88	0.20	5.96	5.96
Soil Organic Carbon	20	1.91	0.32	1.01	0.43
Nitrogen (%)	20	0.16	0.03	0.09	0.04
Phosphorus (mg/kg)	20	9.12	0.09	1.00	1.93
Potassium (mg/kg)	20	173.80	0.70	76.71	46.39
Temperature (°C)	20	19.05	13.13	16.07	1.66
Precipitation (mm)	20	3170.60	796.53	1477.15	652.17

Note: *N = Total number of samples, SD* = Standard Deviation

Juglans regia forest classification

Following Wangda and Ohsawa (2006b) cluster analysis was performed in PC_ORD by taking the RBA of each species from all the plots. Five different forest zones (Forest zone 1 = Cool broad-leaved forest, Forest zone 2 = Mixed conifer forest, Forest zone 3 = Evergreen Oak forest, Forest zone 4 = Cool-dry broad-leaved forest, and Forest zone 5 = Warm moist walnut forest) were classified with a similarity index of 50% (Kaka et al., 2014). Naming of different forest zones were done based on temperature, elevation, rainfall, soil moisture content, and dominant tree species (Ohsawa, 1987).

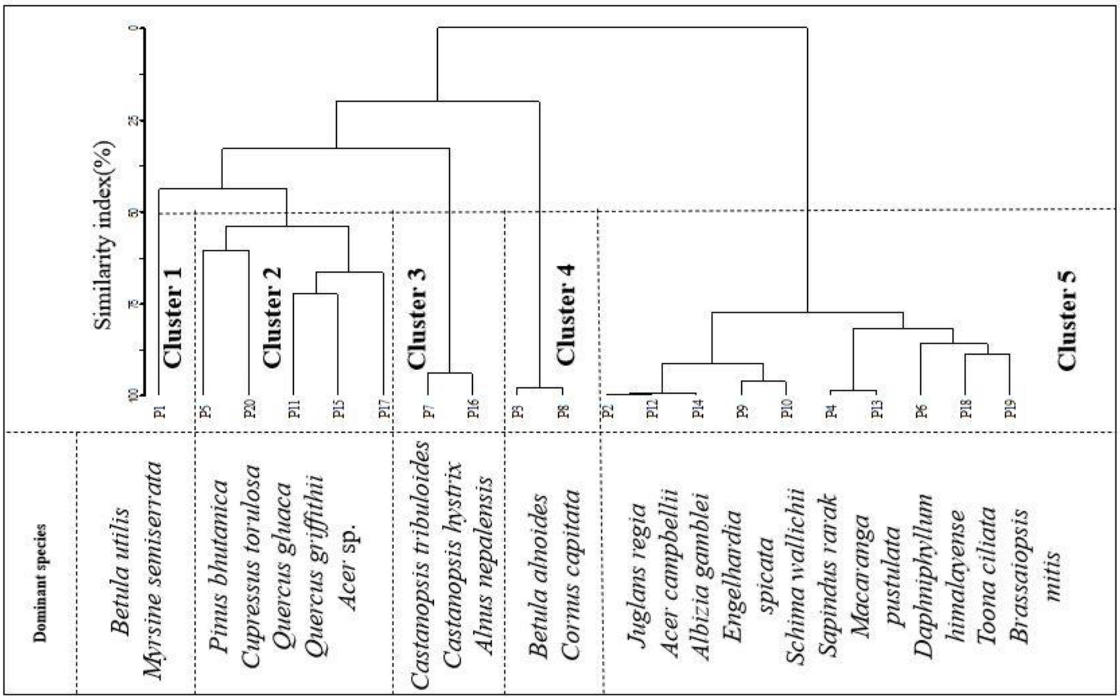


Fig. 4. Cluster Dendrogram of five types of forest classification in *J. regia* forest showing similarity index of 50% and also showing cluster solutions with dotted lines and their possible indicator species

Cluster 1 (Cool broad-leaved forest)

Plot 1 under Gasa district was classified under cool broad-leaved forest owing to its low annual average temperature of 13.13°C compared with the rest of the plots, which had an average annual temperature of 16.07°C (Table 1). The highest elevation (2,594 masl) among all the 20 plots was also noted in this group. The vegetation was dominated by *Betula utilis* and *Myrsine semiserrate* which are usually found growing and thriving better in higher elevation (Shrestha et al., 2007).

Cluster 2 (Mixed conifer forest)

A total of five plots (P5, P10, P11, P15, and P17) were clubbed under this forest zone. The elevation in the plots ranged from 1,586 masl to 2,128 masl. Some of the unique characteristics of these plots were soil MC and soil OM, which were relatively low (5.5 and 1.1 respectively) compared to other plots. One of the driest areas was also noted from this forest type, with plot 8 experiencing as low as 828.312 mm of average annual rainfall (Dorji et al., 2016). The dominating species found in this forest zone were conifer species (*Pinus bhutanica* and *Cupressus torulosa*) and broad-leaved species (*Quercus glauca*, *Quercus griffithii*, and *Acer* sp.) (DoFPS, 2012).

Cluster 3 (Evergreen oak forest)

Two plots (P07 and P16) were clubbed in this zone. The plots were characterized with the presence of similar annual average temperature of 14.4ºC and 16.9°C respectively. Both the plots had same soil N content of 0.09 each. Both the plots represented a transition in vegetation type and were mostly dominated by evergreen trees with the presence of maximum number of species belonging to family Fagaceae (*Castanopsis hystrix* and *C. tribuloides*) (Petit et al., 2013) along with the presence of other tree species such as *Alnus nepalensis,* which were found growing with species belonging to the of family Fagaceae.

Cluster 4 (Cool-dry broad-leaved forest)

The fourth forest Zone comprised two plots (P03 and P08), which were characterized by the presence of low temperature (14.05ºC and 16.39°C), and less rainfall (1,366.13 mm and 823.81 mm) and low soil moisture (6.16% and 4.38%). So, the name cool-dry broad-leaved forest was assigned to this cluster or zone. The dominating species in these plots were *Betula alnoides* and *Cornus capitata.*

Cluster 5 (Warm moist walnut forest)

The fifth and the final forest zone consisted of 10 plots (P02, P12, P14, P09, P10, P04, P13, P06, P18, and P19) and was named warm moist walnut forest, as the main dominating species in these plots was walnut (*J. regia*) with *Acer campbellii, Engelhardia spicata, Schima wallichii, Sapindus rarak, Toona ciliata, Daphniphyllum himalayense, Brassaiopsis mitis, Macaranga pustulata,* and *Albizia gamblei* as the co-dominating species. All the environmental factors reflected by Wani et al. (2016) was found ranging in these 10 plots. The average annual temperature in this forest zone ranged between 14.00ºC–19.05°C with average annual rainfall of 796.52 mm–2,424.39 mm. The highest soil moisture content (27.88%) was also recorded from this cluster favoring the growth of *J. regia* in this cluster.

Influence of environmental variables on the growth and distribution of J. regia

Canonical Correspondence Analysis (CCA) multivariate constrained ordination was used to determine the relationship between the vegetation structure and environmental attributes (Wangda and Ohsawa, 2006a) of plots (Table 2; Fig. 5) where *J. regia* was found. The biplots of CCA ordination clearly reflects that Axis 1 is mostly related with Altitude, soil N, soil K, and Slope and Axis 2 is mostly related with temperature and precipitation.

From the Pearson and Kendall Correlation with ordination axis shown in Table 2, slope (r = 0.66) had the maximum influence on four plots (P06, P07, P08, and P11) with species *Acer sterculiaceum*, *Acer* sp., *Daphniphyllum himalayense*, *Quercus glauca*, *Illicium griffithii*, *Castanopsis tribuloides*, and *Symplocos* *lucida*. Similarly, altitude (*r* = 0.55) had the maximum influence on eight plots (P01, P02, P03 P04, P09, P12, P13, P14) in which *J. regia* was the most dominating species indicating greater influence of altitude on the growth and distribution of *J. regia* in the study area. Some of the species associated with *J. regia* in these plots were *Betula utilis* and *Myrsine semiserrata*, which perform well in higher altitude areas (Shrestha et al., 2007).

Soil N and soil K were also found to have influence (*r* = 0.43 and 0.29) in Plots 06,07,08, and P11 indicating strong influence of the parameter in the growth of the species in those plots (Fernández-Moya et al., 2019). In the ordination plot, soil K had the shortest arrow length (*r* = 0.29) with Pearson Kendall correlation indicating less influence of the parameter on the growth and distribution of the species.

Similarly, ordination biplot showed maximum influence of rainfall in five plots (P16, P17, P18, P19, P20) with strong Pearson and Kendall correlation (*r* = -0.64) in opposite direction indicating negative relation with *J. regia* growth and distribution in these plots. Additionally, temperature had moderate influence (*r* = -0.54) in three plots (P05, P10, P15) in the opposite direction. This indicates that the species has wider adaptation to varying rainfall and temperature. *Juglans regia* is found establishing and growing in a wide range of climatic conditions and is inured to drought being deeply rooted species (Hemery et al., 2010). The species can tolerate drought for a considerable period but rainfall should not fall below 100 mm–150 mm during growing season (Mohni et al., 2009). The species requires a mean annual temperature between 10.5°C to 15°C however the annual mean winter temperature should not fall below 2.5°C (Hemery et al., 2010). Soil parameters such as soil pH and soil P had negligible influence on the growth and distribution of vegetation in the study, thus they were eliminated from the ordination biplot.

Table 2. Pearson and Kendall Correlation with Ordination Axes (N = 20)

Axis	1			2		
	r	r^2	*tau*	*r*	r^2	*tau*
Altitude	0.55	0.30	0.32	0.32	0.10	-0.22
Slope	0.66	0.43	0.50	0.00	0.00	0.02
Temp	-0.54	0.29	-0.41	-0.10	0.01	-0.13
Precipitation	-0.64	0.41	-0.47	-0.41	-0.16	-0.11
Soil pH	0.23	0.05	0.18	-0.01	0.00	0.02
Nitrogen	0.43	0.18	0.31	0.14	0.02	0.13
Soil P	-0.02	0.00	-0.04	0.12	0.01	-0.03
Soil K	0.29	0.09	0.25	-0.29	0.08	0.07

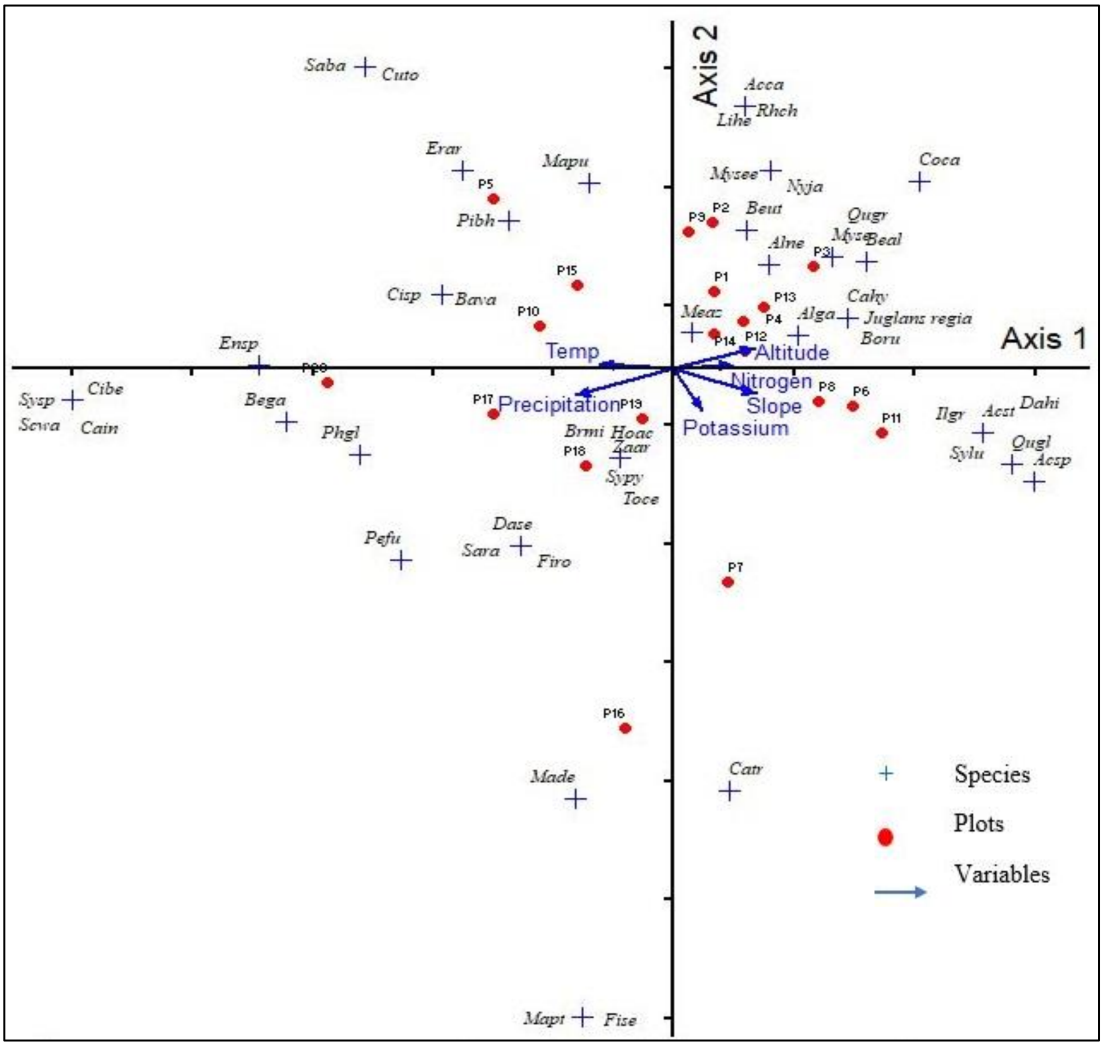


Fig. 5. Canonical Correspondence Analysis (CCA) ordination biplot showing influence of environmental variables on the growth and distribution of *J. regia*

The CCA ordination biplot indicates that the growth and distribution of *J. regia* are influenced by altitude and slope followed by nitrogen indicating better performance, growth, and distribution of the species at higher altitudes within the study area. The findings are in line with the study conducted by Winter et al. (2009) in Southern Kyrgyzstan where it was reported that altitude had a direct influence on the radial growth of *J. regia* and higher altitude provided favorable conditions for the growth of the species when compared to lower altitude. Similarly, a study conducted by Loacker et al. (2007) reported that *J. regia* is greatly influenced by slope and performs well in less sloppy area and is usually found growing up to 60°.

Suitable habitat for the growth of Juglans regia in Bhutan

The environmental factors contributing to the growth and development of *J. regia* in the country were climatic (rainfall and temperature) and topographic (aspect, slope, and elevation) variables collected from the 20 plots.

Using reclassifying tool in ArcGIS, all the raster data was converted in suitable range favoring the growth of the species.

The ArcGIS suitability analysis revealed 18.61% (7,146.53 km^2) of Bhutan's total area as suitable habitat for the growth and development of *J. regia* (Fig. 6). The analysis also revealed that all 20 districts of the country are feasible for growth of the species. Wangdue Phodrang district has the largest suitable habitat for the growth of *J. regia* with a total feasible area of 736.46 km^2 while Bumthang district has the lowest area with about 14.11 km^2.

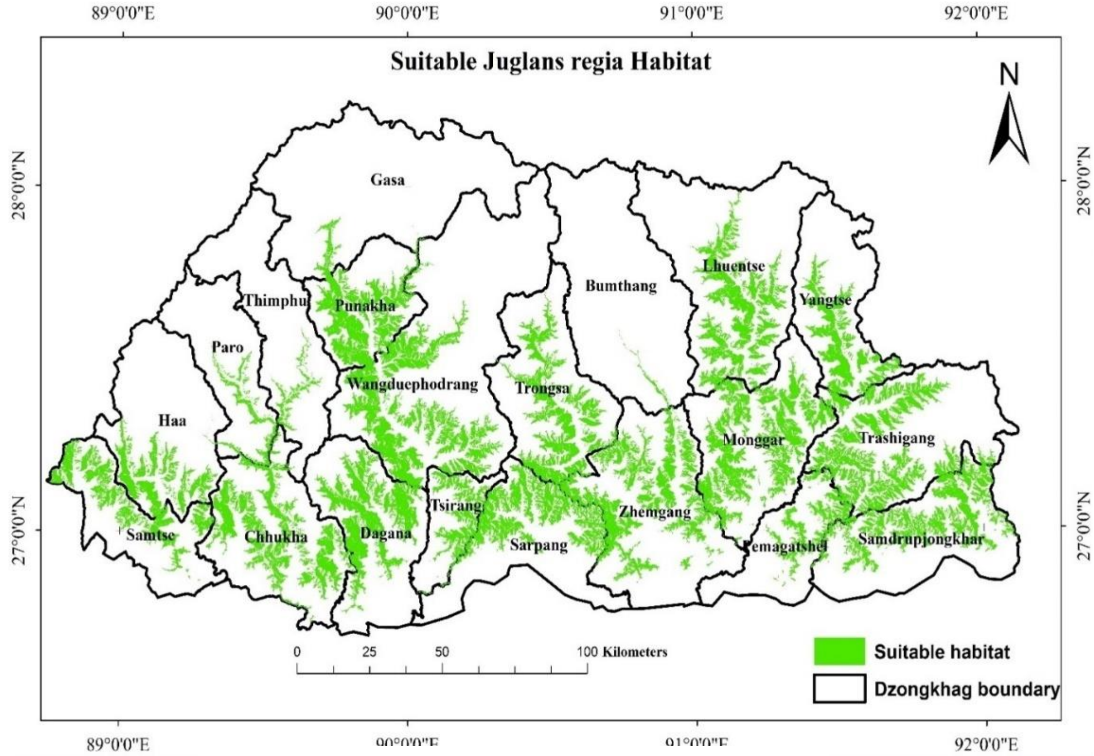


Fig. 6. Suitable habitat of *J. regia* in Bhutan

Conclusions and recommendation

A total of 163 plant species belonging to 74 families were recorded from 20 plots surveyed in four districts. Evergreen species were in abundance under major lifeform trees. The understory (shrub) and groundcover vegetation were dominated by evergreen shrubs and perennial herbs respectively. Growth and distribution of *J. regia* was predominantly influenced by altitude and slope indicating better performance of the species in higher altitude and slope up to 45°. However, temperature and rainfall have negative influence on the growth of *J. regia* indicating greater adaptation in varying climatic condition. The species was mostly found growing in areas with high soil MC and slightly acidic soil. Bhutan has about 18.61% of area suitable for growth of the species, indicating adequate coverage for plantation, protection and management of the species in near future. Through this study we could understand the basic growth requirement of the species covered in a relatively small area with a smaller number of plots. The current study could cover only four districts and relatively less altitudinal range. Further study covering larger area and greater altitudinal range with more samples is required. The study was carried out during winter and additional studies in different seasons can be done to fully understand the floristic ecology.

Authors contribution statement

Mr. Laxmi Sagar, Mr. Ngawang Gyeltshen, Mr. Bhakta Bdr. Ghalley, Mr. Sonam Younten, Mr. Namkha Gyeltshen and Mr. Rupesh Subedi were involved in developing general idea, study design, data collection, analysis and manuscript drafting. Mr. Jambay was also involved in study design while his main contribution was on editing the contents.

Conflict of interest statement

None

Acknowledgements

The authors would like to thank Department of Forest and Park Services for granting clearance to carry out the research. The authors acknowledge *IDEA WILD* for equipment support. We are grateful to the Chief of JDNP and Divisional Forest office Dagana and Wangdue for all their support rendered during the study. Similarly, special gratitude is for Mr. Norbu Wangdi (Forest Ranger, Wangdue Phodrang Range), Mr. Tashi (Forest Ranger, JDNP), and Mr. Binod Alley and Mr. Moni Kumar Neopani (Forest Ranger, Drujeygang Range) for all the technical and logistic support.

References

Acharya, A. S., Prakash, A., Saxena, P., and Nigam, A., 2013. Sampling: why and how of it? *Indian Journal of Medical Specialities*, 4(2): 330–333. https://doi.org/10.7713/ijms.2013.0032

Arab, M. M., Marrano, A., Abdollahi-Arpanahi, R., Leslie, C. A., Askari, H., Neale, D. B. and Vahdati, K., 2019. Genome-wide patterns of population structure and association mapping of nut-related traits in Persian walnut populations from Iran using the Axiom J. regia 700K SNP array. *Scientific Reports*, 9(1): 1–14. https://doi.org/10.1038/s41598-019-42940-1

Behrooz, A., Vahdati, K., Rejali, F., Lotfi, M., Sarikhani, S., and Leslie, C., 2019. Arbuscular mycorrhiza and plant growth-promoting bacteria alleviate drought stress in walnut. *HortScience*, *54*(6): 1087–1092. https://doi.org/10.21273/HORTSCI13961-19

Bhattarai, B. P., and Tomar, C. S., 2009. Effect of Integrated Nutrient Management on Leaf Nutrient Status of Walnut (Juglans regia L.). *Nepal Journal of Science and Technology*, 10(1): 63–67.

Bremner, J. M., 1960. Determination of nitrogen in soil by the Kjeldahl method. *Journal of Agricultural Sciences*, 55(1): 11-13. https://doi.org/10.1017/S0021859600021572.

Bray, R.H. and Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59: 39–45

Burtin, P., Jay-Allemand, C., Charpentier, J. P., and Janin, G., 1998. Natural wood colouring process in Juglans sp. (J. nigra, J. regia and hybrid J. nigra 23 x J. regia) depends on native phenolic compounds accumulated in the transition zone between sapwood and heartwood. *Trees - Structure and Function*, 12(5): 258–264. https://doi.org/10.1007/PL00009716

Chen, F., Chen, J., Wang, Z., Zhang, J., Li, X., Lin, M., Song, Y., and Zhang, L., 2019. Genomics: cracking the mysteries of walnuts. *Journal of Genetics*, 98(2): 1–3. https://doi.org/10.1007/s12041-019-1084-3

Cheng-jun, J. I., Yuan-he, Y., Wen-xuan, H. A. N., Yan-fang, H. E., Smith, J., and Smith, P., 2014. Climatic and Edaphic Controls on Soil pH in Alpine Grasslands on the Tibetan Plateau , China : A Quantitative Analysis. *Pedosphere*, 24(1): 39–44. https://doi.org/10.1016/S1002-0160(13)60078-8

Claassen, V. P., and Carey, J. L., 2013. Regeneration of Nitrogen Fertility In Disturbed Soils Using Composts. *Compost Science & Utilization*, 12(2): 145–152.

Clay, D. E., Kitchen, N., Carlson, C. G., Kleinjan, J. L., and Tjentland, W. A., 2002. Collecting Representative Soil Samples for N and P Fertilizer Recommendations. *Crop Management*, 1(1), 1–5. https://doi.org/10.1094/cm-2002-1216-01-ma

Cosmulescu, S., Baciu, A., Botu, M., and Achim, G., 2010. Environmental factors' influence on walnut flowering. *Acta Horticulturae*, *861*: 83–88. https://doi.org/10.17660/ActaHortic.2010.861.10

DoA., 2019. *Package of Practices for field and horticulture crops of Bhutan*. Agriculture Research and Extension Division (ARED), Ministry of Agriculture and Forests, Thimphu, Bhutan.

DoF., 2006. *Royalty on Forest Produce*. Ministry of Agriculture, Department of Forest, Thimphu, Bhutan.

DoFPS., 2012. *Field Manual: National Forest Inventory of Bhutan*. Department of Forest and Park Services, Ministry of Agriculture and Forest, Thimphu, Bhutan.

DoFPS., 2020. Biodiversity Monitoring and Social Survey Protocol of Bhutan. Nature Conservation Division, Department of Forests and Park Services, Ministry of Agriculture and Forests, Royal Government of Bhutan, Thimphu Bhutan.

DoFPS., 2021. *Jigme Dorji National Park*. <http://www.dofps.gov.bt/?page_id=162>. Accessed 19 January 2021.

Dökmen, F., Ahi, Y., and Köksal, D. D., 2017. Irrigation of walnut trees in the vicinity of Hisareyn - Gölcük / Kocaeli, Turkey. *European Water*, 59: 339–342.

Dong, W., Xu, C., Li, W., Xie, X., Lu, Y., Liu, Y., Jin, X., and Suo, Z., 2017. Phylogenetic resolution in juglans based on complete chloroplast genomes and nuclear DNA sequences. *Frontiers in Plant Science*, 8(1148): 1-13. https://doi.org/10.3389/fpls.2017.01148

Dorji, U., Olesen, J. E., Bøcher, P. K., and Seidenkrantz, M. S., 2016. Spatial variation of temperature and precipitation in Bhutan and links to vegetation and land cover. *Mountain Research and Development*, 36(1): 66–79. https://doi.org/10.1659/MRD-JOURNAL-D-15-00020.1

Fernández-Moya, J., Urbán-Martínez, I., Pelleri, F., Castro, G., Bergante, S., Giorcelli, A., Gennaro, M., Licea-Moreno, R., Santacruz Pérez, D., Gutiérrez-Tejón, E., HomarSánchez, C., Bidini, C., Chiarabaglio, P., Manetti, M., Plutino, M., and Sansone, D., 2019. *Silvicultural guide to managing walnut plantations for timber production*. European Commission, Bosques Naturales, S.A.

FRMD., 2017. *Land Use and Land Cover of Bhutan, Maps and Statistics*. Forest Resources Management Division, Department of Forests & Park Services, Ministry of Agriculture and Forests, Thimphu, Bhutan.

Gauthier, M. M., and Jacobs, D. F., 2011. Walnut (Juglans spp.) ecophysiology in response to environmental stresses and potential acclimation to climate change. *Annals of Forest Science*, 68(8): 1277–1290. https://doi.org/10.1007/s13595-011-0135-6

Ghimiray, M., and Katwal, T. B., 2013. Crop Genetic Resources for Food Security and Adaptation to Climate Change: A Review and Way Forward. *Journal of Renewable Natural Resources Bhutan*, 9(1): 1–14.

Griersin, A. J. ., and Long, D., 1983. *Flora of Bhutan: Including records of plants from Sikkim: Volume 1 Part 1*. Royal Botanical Garden, Edinburgh.

Guo, W., Chen, J., Li, J., Huang, J., Wang, Z., and Lim, K. J., 2020. Portal of Juglandaceae: A comprehensive platform for Juglandaceae study. *Horticulture Research*, 7(1): 1-8. https://doi.org/10.1038/s41438-020-0256-x

Hemery, G. E., Clark, J. R., Aldinger, E., Claessens, H., Malvolti, M. E., O'connor, E., Raftoyannis, Y., Savill, P. S., and Brus, R., 2010. Growing scattered broadleaved tree species in Europe in a changing climate: a review of risks and opportunities. *Forestry*, 83(1): 65–81. https://doi.org/10.1093/forestry/cpp034

Jeyakumar, S. P., Dash, B., Singh, A. K., Suyal, D. C., and Soni, R., 2020. Nutrient Cycling at Higher Atitude. In R. Goel, R. Soni, & D. C. Suyal (Eds.), *Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability* (p 293-305). Springer Singapore.

Kaka, Gurung, D. B., Khandu, Y., Katel, O., Chhetri, P.B., and Wangda, P., 2014. Population Structure of Cupressus tortulosa Griffith along the Altitudinal Gradients of Tsendenanag Ridge in Punakha, Bhutan. *Bhutan Journal of Natural Resources and Development*, 1(1): 24–31. 10.17102/cnr.2014.04

Kenkel, N. and Podani, J., 1991. Plot size and estimation efficiency in plant community studies.
Journal of Vegetation Science, 2(4): 539–544.

Knudsen, D., Peterson, G., and Pratt, P., 1982. Lithium, Sodium, and potassium. In A. L. Page (Eds.), *Method of Soil Analysis* (pp. 225–246). American Society of Agronomy, Madison, USA.

Loacker, K., Kofler, W., Pagitz, K., and Oberhuber, W., 2007. Spread of walnut (Juglans regia L.) in an Alpine valley is correlated with climate warming. *Flora: Morphology, Distribution, Functional Ecology of Plants*, 202(1): 70–78. https://doi.org/10.1016/j.flora.2006.03.003

Manos, P. S., and Stone, D. E., 2001. Evolution, phylogeny, and systematics of the Juglandaceae.

Mariana, B., and Niculina, C. S., 2017. Effect of Climatic Conditions on Flowering of Walnut Genotypes in Romania. *Journal of Nuts*, 8(2): 161–167.

McGranahan, G., and Lesli, C., 2009. Breeding Walnuts. In P.M. Priyadarshan & S.M. Jain (Eds.), *Breeding Plantation Tree Crops: Temperate Species* (pp. 249–273). Springer New York, USA. https://doi.org/10.1007/978-0-387-71203-1

Mohni, C., Pelleri, F., and Hemery, G. E., 2009. The modern silviculture of Juglans regia L.: A literature review. *Die Bodenkultur*, 60(3): 21–34.

Molnar, T., 2011. Persian Walnuts (Juglans regia L.) in Central Asia. *Annual Report of the Northern Nut Growers Association*, 101, 56–69.

NSB., 2021. *Agriculture Survey Report*. National Statistics Bureau, Royal Government of Bhutan, Thimphu, Bhutan.

NHMC., 2021. *Bhutan State of the Climate 2020.* National Center for Hydrology and Meterology, Royal Government of Bhutan, Thimphu Bhutan, p.38. https://www.nchm.gov.bt/

Ohsawa, M., 1987. Life zone ecology of the Bhutan Himalaya. Laboratory of Ecology, Chiba University, Japan.

Pakale, A. A., TanajiJadhav, P., and Jadhav, P., 2018. Digital pH meter. *Journal of Electronic Design Engineering*, 4(1): 1–4. https://doi.org/10.1016/s0026-0576(04)90035-9

Petit, R. J., Carlos, J., Curtu, A. L., Loustau, M.L., Plomion, C., Gonzalez-Rodrıguez, A., . . . Ducousso, A., 2013. Fagaceae trees as models tointegrate ecology, evolution and genomics. *New Phytologist*, 197 (2): 369-371. https://doi.org/10.1111/nph.12089

Quermé, A., Battie-laclau, P., Dufour, L., Wery, J., and Dupraz, C., 2017. Effects of walnut trees on biological nitrogen fixation and yield of intercropped alfalfa in a Mediterranean agroforestry system. *European Journal of Agronomy*, 84(1): 35–46. https://doi.org/10.1016/j.eja.2016.12.001

Rabgay, T., Gurung, D., Jambay, J., Wangchuk, K., Thinley, P., Tshering, B., Penjor, P., Dorji, T., Tshering, K., Sitaula, B., and Raut, N., 2020. Environmental Factors Affecting Growth of Agarwood (Aquilaria malaccensis Lamarck, 1783) Forests of Bhutan. *Bhutan Journal of Natural Resources and Development*, 7(1): 12–25. https://doi.org/10.17102/cnr.2020.42

Reuter, H. I., Lado, L. R., Hengl, T., and Montanarella, L., 2008. Continental-Scale Digital Soil Mapping using European Soil Profile Data: Soil pH. In J. Böhner, T. Blaschke, & L. Montanarella (Eds.), *Hamburgr Contributions to Physical Geography and LandscapeEcology* (pp. 91–102). Universität Hamburg Germany.

Rao, P., Barik, S. K., Pandey, H. N. and Tripathi, R. S., 1990. Community composition and tree population structure in a sub-tropical broad-leaved forest along a disturbance gradient. *Vegetation*, 88(2): 151-162.

RGoB., 2018a. Dagana Dzongkhag Administration. <http://www.dagana.gov.bt/about-district>. Accessed 19 January 2021.

RGoB., 2018b. Gasa Dzongkhag Administration. <http://www.gasa.gov.bt/about-district>. Accessed 19 January 2021

RGoB. 2019. Punakha Dzongkhag Administration. <http://www.punakha.gov.bt/about-dzongkhag>. Accessed 19 January 2021.

Rigo, de D., Enescu, C, M., Durrant, Houston, T., Tinner, W. and Caudullo, G., 2016. Juglans regia in Europe: distribution, habitat, usage and threats. In J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, T. Houston Durrant & A. Mauri (Eds.), *European Atlas of Forest Tree Species*, p.103). Publication Office of the EU, Luxembourg. 10.2788/038466

Shrestha, B.B., Ghimire, B., Lekhak, H.D., and Jha, P.K., 2007. Regeneration of Treeline Birch (Betula utilis D . Don) Forest in a Trans-Himalayan Dry Valley in Central Nepal. *Mountain Research and Development*, 27(3): 259–267. https://doi.org/10.1659/mrdd.0784

Slessarew, E. W., Lin, Y., Bingham, N. L., Johnson, J. E., Dai, Y., Schimel, J. P., and Chadwick, O. A., 2016. Water balance creates a threshold in soil pH at the global scale. *Nature*, 540: 567–569. https://doi.org/10.1038/nature20139

Teketay, D., 2005. Seed and regeneration ecology in dry Afromontane forests of Ethiopia: Seed production - Population structures. *Tropical Ecology*, 46(1): 29–44.

Tesfaye, G., Teketay, D., Fetene, M., and Beck, E., 2010. Regeneration of seven indigenous tree species in a dry Afromontane forest, southern Ethiopia. *Flora: Morphology, Distribution, Functional Ecology of Plants*, 205(2): 135–143. https://doi.org/10.1016/j.flora.2008.12.006

Thakur, A. (2011). Juglone: A therapeutic phytochemical from Juglans regia L. *Journal of Medicinal Plant Research*, *5*(22): 5324–5330.

Vahdati, K., 2014. Traditions and Folks for Walnut Growing around the Silk Road. *Acta Horticulturae*, 1032: 19–24.

Vahdati, K., Lotfi, N., Kholdebarin, B., Hassani, D., Amiri, R., Mozaffari, M. R., and Leslie, C., 2009. Screening for drought-tolerant Genotypes of persian walnuts (Juglans regia L.) during seed

Germination. *HortScience*, 44(7): 1815–1819. https://doi.org/10.21273/hortsci.44.7.1815

Wangda, P., and Ohsawa, M., 2006a. Gradational forest change along the climatically dry valley slopes of Bhutan in the midst of humid eastern Himalaya. *Plant Ecology*, 186(1): 109–128. https://doi.org/10.1007/s11258-006-9116-5

Wangda, P., and Ohsawa, M., 2006b. Structure and regeneration dynamics of dominant tree species along altitudinal gradient in a dry valley slopes of the Bhutan Himalaya. *Forest Ecology and Management*, 230(1–3): 136–150. https://doi.org/10.1016/j.foreco.2006.04.027

Wani, M. S., Hussain, A., Ganie, S. A., Munshi, A. H., Lal, E. P., and Gupta, R. C., 2016. Juglans Regia – a Review. *International Journal of Latest Research in Science and Technology ISSN*, 5(1), 2278–5299. http://www.mnkjournals.com/ijlrst.htm

Winter, M. B., Wolff, B., Gottschling, H., and Cherubini, P., 2009. The impact of climate on radial growth and nut production of Persian walnut (Juglans regia L.) in Southern Kyrgyzstan. *European Journal of Forest Research*, 128(6): 531–542. https://doi.org/10.1007/s10342-009-0295-1

Zohary. D., Hopf, Maria., and Weiss, E.,2012. Domestication of Plants in the Old World: *The origin and spread of domesticated plants in south-west Asia, Europe, and the Mediterranean Basin (Fourth Edition).* Oxford University Press, England, p. 269, ISBN: 978–0–19–954906–1