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A marvelous hide and speculation in the life of *Rhododendron* species at a glance

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Abstract
Rhododendron is a multi-quality, astonishing rose tree, having aristocratic role in human and animal's life. This largest genus belongs to family *Ericaceae* and largest flowering plant of Asia. This genus having 1025 species and 10,000 of varieties worldwide and 571 species recorded in China, 155 endemic species in New Guinea, 16 at Russian Federation and 80 species in India. It grown in drained acidic soil in higher concentration in Eastern Himalayas. The soil, humidity, rainfall, light, temperature, altitude, slope and atmospheric pressure are the growth augmentation factors. Flowering occurs first at lower altitude during March-April. The altitude height shows negative correlation with the flower colour. Geitonogamous type of pollination produces highest fruit setting where self-pollination shows self-compatibility. The diploid species contain $2n = 2x = 26$ and tetraploid $2n = 4x = 52$ chromosome number. Due to global warming *R. arboreum* shows precautious flowering because of average temperature increases where ultraviolet rays increase in atmosphere which is responsible for early flowering in mountain plants. The genome mapping and identification and development of QTLs through DNA markers would make a revolution for the conservation and protection. Therefore it is necessary to encourage the traditional, scientific and molecular technologies and knowledge for improving the growth pattern of *Rhododendron species.*

Keywords: *Rhododendron*, geitonogamous, QTLs, genome mapping and global warming

Introduction
Rhododendron arboreum is an evergreen shrub or small tree producing bright red flowers. The "Rhododendron" name was derived from the Greek word 'Rhodo' means rose and "Dendron" means tree as combination 'rosetree' (Hora, 1981) [42]. It is known by several names like Burans, Bras, Buras and Barahke Phool in Uttarakhand. Rhododendron is a national flower and state flower of Nepal and Uttarakhand respectively. It is known by other vernacular names such as Lali gurans (Nepali), Adrawal (Punjabi), Billi (Tamil), Pu (Kannada) and Kattupoo varasu (Malayalam) languages (Srivastava, 2012) [108]. This genus is economically important as a ornamental plants due to its beautiful and diverse colour ans shape of flower behavior (Paul *et al.*, 2005, Gibbs *et al.*, 2011) [83, 38]. *Rhododendron arboreum* a Himalayan species populates the bottom canopy of forests, alongside pine and oak, where the temperature ranges from 20 °C to as low as −10 °C surrounding an environment (Choudhary *et al.*, 2019) [20].
It was described first time by Carl Linnaeus in 1737 in Genera Plantarum (Singh, 2009) [104]. *Rhododendron arboreum* is an impressive and stately species of rhododendron and extremely variable in stature, hardness, flower colour and leaf characteristics and this species arboreum means tree like (Orwa *et al.*, 2009, Srivastava, 2012) [80, 108]. The largest genus of rhododendron belongs to family *Ericaceae* as well as among one of the largest flowering plant genera in Asia (Cullen and Chamberlain, 1978) [27].
The genus *Rhododendron* habitat rages from subtropical and temperate to subalpine and alpine ecosystems. Maximum species diversity about 48 species recorded from the temperate and subalpine regions of Himalayas between 3000-3500 m (Bhattacharyya and Sanjappa, 2008) [10]. Most of the rhododendrons are found in high rainfall humid temperate regions where highly organic well drained acidic soil presents (Milleville, 2002) [69]. The acidic soil created by the degradation of acidic liter of *R. arboreum* (Maithani *et al.*, 1998) [62]. Mosses and lichens are commonly associated with the rhododendron species. Eight species are found growing epilithically/epiphytically, which are considered as confined to eastern Himalayas and

north-east India. Such kind of species are entirely absent in western Himalayas (Bhattacharyya and Sanjappa, 2008) [10]. Other types of rhododendron species are presented in table 2. Based on the different uses of the *Rhododendron* we need to conserve and protect it to become an endangered species. Somewhat, some of the species are near to verge of extinction due to urbanization and industrialization and other climatic factors (Global warming). The *Ex- situ* conservation of rhododendrons have been also initiated in India by G. B. Pant Institute of Himalayan Environment and Development at its arboretum near Pangthang, Sikkim at *c*. 1800 m altitude where 24 species are grown (Singh *et al*., 2003) [102].

Distribution

The genus *Rhododendron* is represented about 1025 species globally (Chamberlain *et al.,* 1996) [16] but according to Jing *et al.,* (2015) [45] this genus contains 1000 species. And over 10,000 varieties word wide (Zaytseva *et al.,* 2018) [130]**.** The rhododendron distributed from the northern temperate zone, throughout tropical southeastern Asia, to north-eastern Australia (Chamberlain, 1996) [16]. There are 562 species have been recorded in China, of which 405 species are endemic, and a large part of this diversity is represented by subgenus *Hymenanthes* (Blume) K. Koch, which comprises about 24 subsections with 225 species, which occurs at China in majority (Chamberlain, 1982, Fang and Min, 1995) [17, 33]. The genus mostly present in the temperate regions of northern hemisphere especially, in Sino-Himalayas (Eastern Himalayas and Western China) (Bhattacharyya and Sanjappa, 2008) [10].

According to Mingyuan *et al*., (2005) [70] there are about 571 species of rhododendron are found in China. It is also found that in good number at Myanmar, Thailand, Malaysia, Indonesia, Philippines and New Guinea. In New Guinea there are 155 species are endemic (Perhaps a secondary center of distribution). From Afghanistan, Pakistan, southern Europe and northern America a few number of species have been reported. Only two species are reported from Australia. There are no rhododendrons present in Africa, central and southern America which is a grotesque fact (Hutchinson, 1947) [43]. Sixteen *Rhododendron* species occurs in Russian Federation and 13 of them occurs only in Siberia and the Russian Far East (Budantsev, 2009) [14]. There are about 80 species with 10 subspecies and 14 varieties have been reported in India (Bhattacharyya, 2005) [11]. Out of these 80 species, there are 21 can be considered as rare in India (Bhattacharyya and Sanjappa, 2008) [10].

In India it is widely distributed in different regions and altitudes mainly in the Himalayas. There are five climatic zones on the basis of altitudes such as 1) warm temperate (900-1800 m), cool temperate (1800-2400 m), cold zones (2400-3000 m), alpine zone (3000-4000 m), glacier zone (4000-4800 m), and frozen zone (above 4800 m) (Uttarakhand Forest Statistics 2012-2013) [120]. The physiographic zones are characterized into two broad categories: i) non-montane zone consisting of 'bhabhar' (Surface of Shivalik foothills), and 'tarai region' (Marsh damp) areas and ii) montane zone including sub-Himalayas (Shivalik hills and Doon valleys), mid-Himalayas (Lesser/ Lower/ Little/ Himanchal/ Mahabharat range Himalaya), greater-Himalaya (Higher Himalayas/'Himadri') and trans-Himalaya (Tibetan/ Tethys Himalaya) (Uttaranchal State of Environment, 2004) [121].

The four montane zones of Himalays, the rhododendron practically absent in Shivalik region, few are found in lesser Himalayas and present in majority of them are in the greater Himalayas. Only one species occurs in trans-Himalayas region, which is located in extreme north-west India (Including cold desert of Jammu and Kashmir and Himanchal Pradesh). For the Ideal locality the greater Himalayas is perfect for rhododendron in India. Besides this in the north-eastern region of India a good number of species are found particularly in Naga and Khasi hills. The only species *Rhododendron arboreum* spp. *nilagiricum* occurs in western Ghats (Bhattacharyya and Sanjappa, 2008) [10].

Description

Rhododendron taxonomically classified such as Kingdom-Plantae, Phylum-Magnoliophyta, Class-Angiospermae, Order-Ericales, Family-Ericacease, Genera- *Rhododendron* and Species- *arboreum*. There are some subspecies of *Rhododendron- arboreum* like 1) *Rhododendron arboreum spp. arboreum* (Red or rose red flowers) found in Western India, 2) *Rhododendron arboreum spp. cinnamomeum* (white, pink or red flowers) found in Central Himalays, 3) *Rhododendron arboreum* spp. *delavayii* (Red flower) found in Eastern Himalays, 4) *Rhododendron arboreum spp. nilagiricum* (Red flower) found in Nilgiri and 5) *Rhododendron arboreum spp. zeylancium* (Orange red flowers) found in Sri Lanka (Srivastava, 2012) [109]. A one species of *Rhododendron arboreum* flower morphology has been presented in figure 1 and plant morphology presented in table 3. *Rhododendron species* are considerably vary in flower colour as well as number and length of stamens, its occasionally showing dimorphism in stamen length within one flower (Escaravage and Wagner, 2004) [32].

Table 1: Number of Rhododendron species present in the different states of India

S.N.	State	Maximum species present
1.	Arunachal Pradesh	67
2.	Sikkim	36
3.	Darjeeling district of west Bengal	19
4.	Nagaland	07
5.	Manipur	05
6.	Mizoram	02
7.	Meghalaya	02
8.	Uttarakhand	06
9.	Himanchal Pradesh	04
10.	Jammu and Kashmir	04
11.	Tamil Nadu	01
12.	Kerala	01

Source: Bhattacharyya and Sanjappa, (2008) [10].

Table 2: Different types of rhododendron species are present in different areas based on their characteristics

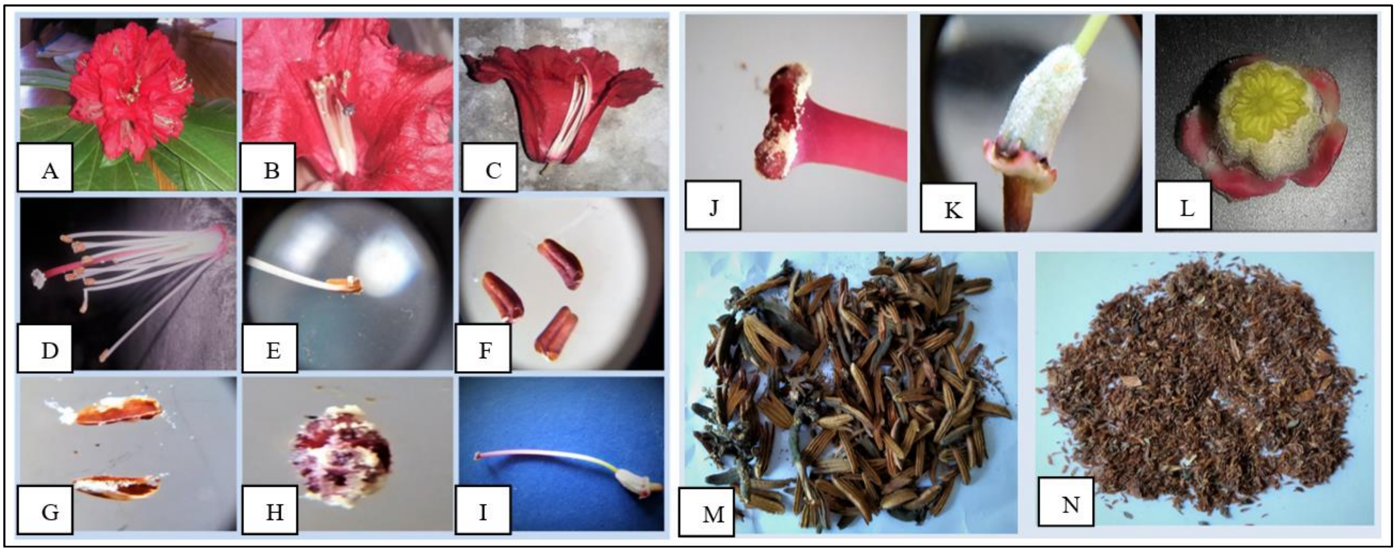
S. N.	Types of species	Area present
1.	Epiphytic species	Eastern Himalayas and north east India, entirely absent in western Himalayas
2.	Arboreal species	Broad leaved forest in subtropical regions, sometimes in pure ericaceous forest
3.	Shrubby species	Temperate forest (between 2700-2900 m), subalpine region (between 2900-3500 m), associated ith *Abies, Tsuga, Betula etc.*
4.	Associated with chir pine (*Pinus roxburghii*)	Western Himalayas and Eastern Arunachal Pradesh
5.	Alpine Rhododendron shrubs	Above 3500 m, dominant between 4200-4600 m
6.	*Rhododendron nivale*	Above 5000 m, sometimes near to glacier

Source: Bhattacharyya and Sanjappa, (2008) [10]

Table 3: Rhododendron pharmacognosy description

S.N.	Plant Part	Characteristics	Source
1.	Trunk	Often much branched, crooked or gnarled	Orwa *et al.*, (2009) [80]
2.	Bark	Reddish brown, soft and rough, exfoliating in thin flakes	Chauhan, (1999) [19]
3.	Leaves	Oblong, lanceolate, 10-20 cm long, 3.6 cm wide, crowded towards the ends of branches, petiole covered with white scales when young	Orwa *et al.*, (2009) [80]
		Glossy green, with deeply impressed veins from above white fawn, cinnamon or rusty brown felt at under surface	Rai and Rai, (1994) [87]
4.	Flower	*Rhododenderon arboreum* range in colour from deep scarlet to red with white, bearing upto twenty blossoms in a single truss, spectacular sight when in full bloom bright red form generally found at the lower elevation	Orwa *et al.*, (2009) [80]
		Flowers are showy, red in dense globose cyme	Chauhan, (1999) [19]
		Calyx fine cleft, corolla tube spotted funnel shaped, stamens hypozygnous declining, filaments filiform, anthers ovate and style capitate	Paxton, (1834) [85]
5.	Fruit	Capsule curved central colum composed of fine lobes, ribbed upto 3.8 cm long and 1.25 cm wide	Orwa *et al.*, (2009) [80]
6.	Seed	Minute seeds, dark brown in colour, compressed thin linear having obvolute membrane	Orwa *et al.*, (2009) [80]

Source: Srivastava, (2012) [109]



A: Inflorescence, B&C: Flower with satmens and stigma, D: stamen with style and stigma in whorl, E&F: Anther, G: Half portion of anther with poolen grains, H: upper portion of stigma, I: gynoecium with style, J: Stigma with pollen grains, K: Feathery gynoecium, L: Vertical section cut of gynoecium, M: Seeds with seed coat and N: Seeds

Fig 1: Visual and microscopic study of flower parts of *Rhododendron arboreum*

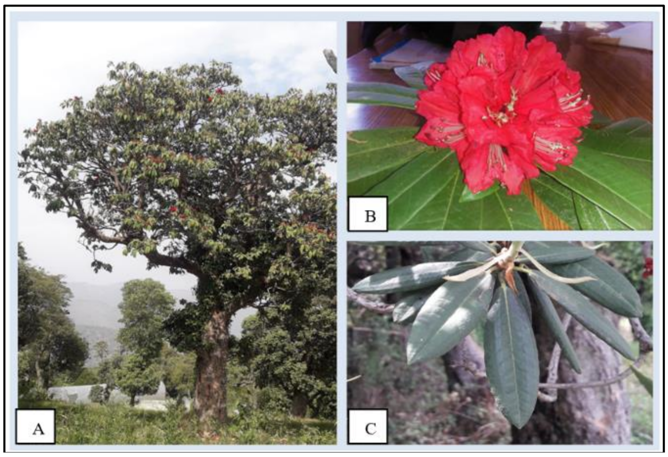


Fig 2: A: *Rhododendron* tree, B: Infloresence and C: Leaves

Growth augmentation factors

There are several growth augmentation factors where the rhododendron can survive and thrive well such as soil, humidity, rainfall, light, temperature, altitude, slope and atmospheric pressure and wind velocity etc. *Rhododendron* grows in well drained acidic soil which is rich in organic matter and low in elements, and no water logged conditions where species grown in slopes. Species including *R. hodgsonii* and *R. falconeri,* etc., are found in swampy soils, Epilithic/epiphytic species are grown either on heavily moist mossy rocks or pendulous from or tree trunks. Alpine species such as *R. anthopogon, R. lepidotum, R. setosum* and *R. nivale* grow in soil with limited water and nutrients. In alpine pastures, all these species when buried under snow during winter and snow melts in summer, when the soil is exposed for a period of 3-4 months the plants start sprouting and flowering. The soil of the subtropical regions of the Himalayas and Western Ghats remains free from snow where *R. arboreum* grows copiously (Bhattacharyya and Sanjappa, 2008) [10].

The light is also an important factor for every plant for photosynthesis, flowering and other morpho-physiological activity. The alpine meadows (Above 3000 m) are relatively shiny as compare to that of subtropical Himalayas. Rhododendrons of these areas are dwarf and stunted habit. Duration of light period in Himalayas region are severely affected between 1500-3000m because of at the flowering time the formation of fog and clouds. This cloud affected areas are broad leaved species grown such as *R falconeri*, *R. grande*, *R. hodgsonii*, *R. sinogrande,* etc. Some of the smaller leaved species like *R. barbatum*, *R. campylocarpum*, *R. thomsonii,* etc. are associated with the above said species. Species occurring in the fogy or cloudy areas (below 3000 m) showed broader and longer leaves as compare to that of occurring above 3000 m. The north and north east areas where hills are exposed the rhododendron s growth is bountiful (Bhattacharyya and Sanjappa, 2008) [10].

Temperature affects the growth and flowering of rhododendron. *R. arboreum* subsp. *arboreum* var. *arboreum* from Lachung (Altitude *ca* 3000 m, temperature recorded 8 °C- 12 °C during day and 1°-3 °C during night in the month of April), North Sikkim was introduced in the nursery of Indian Botanic Garden (IBG), Howrah (Sea level) grew to *c*. 10 cm in 5 years where the average temperature ranges from 25°-33 °C. Another plant of *R. falconeri* from Varsey Rhododendron Sanctuary (2900 m, temperature recorded 12°-15 °C during day, 1 °C - 5 °C during night in the month of April) West Sikkim which was introduced in the Garden of Sikkim Himalayan Circle (*ca* 1800 m, average temperature ranges from 15°-23 °C), Botanical Survey of India, Gangtak whose growth rate was also very slow. The species *R. formosum* (introduced from Elephant falls, upper Shillong, 1960 m, temperature recorded at day time 12°-15 °C during late March) is somewhat heat tolerant which flowered in the gardens of Eastern Circle, Botanical Survey of India, Shillong (1550 m), where the average day temperature recorded 19°-21 °C at the beginning of April. In *R. griffithianum* the flowering occurs in slightly warmer condition in comparison to other species (Bhattacharyya and Sanjappa, 2008) [10]. A curling character of leaves is a natural mechanism during cold weather not only to reduce water loss but also to shed snow (Cox, 1990) [24].

The altitude is obviously related to rhododendron habitat. The higher elevation and wing can make the species dwarf. Those species occurs in higher altitudes they have often thicker indumentums as compare to that of occurring in lower elevations (Cox, 1990) [24]. The duration between flowering and seed ripening is directly relates to the altitudes of a particular species. Generally those plants occurs in higher elevation the seed ripening takes less time in comparison to lower altitudes (Hooker, 1854) [41]. There may be a possibility of early maturity of seeds where some factors are responsible such as alternate wetting, drying and freezing day after day in frosted environment (Kingdon-Ward, 1934) [52]. Other important factor is slope which directly bearing species diversity. Some species like *R. falconeri*, *R. grande*, *R. hodgsonii*, *R. maddenii* (When terricolous), *R niveum*, *etc.*, which prefers to grow in flat ground. Whereas, others species such as *R. formosum*, *R. johnstoneanum*, *etc*. like growing in ravines and on slopes. This factor is directly related to the water content of the soil. Most of the species prefers to grow in well drained soils thus they are called slope loving species. Maximum number of species occurs in south facing slopes because of much availability of light and moisture. Other factor is atmospheric pressure and velocity of wind. Due to low atmospheric pressure and high velocity of wind causes desiccation of *Rhododendron* in alpine zones, which in turn the modification takes place in growth form into dwarf shrubs with stunted or prostrate woody branches (Bhattacharyya and Sanjappa, 2008) [10].

Flowering impediment

The *Rhododendron* tree performs short in height if grown near to villages or agricultural fields as compare to that of trees found in forest. In *R. arboreum* flowering occurs first in lower altitude and gradually progress into higher altitude area. It was found that the flowering opening inducting in the 3rd week of March, however low altitude areas it is now occurring during the first to second weeks of March. Generally in a particular year the flowering showed copious behavior whereas comparatively less in the subsequent year. The altitudes height and flower colour showed negative correlation. If the altitude increases, the flower colour becomes lighter. The red flower colour appears in lower altitude (1700-2100 m) but as the altitude increases the flower clour becomes dark pink, pink, light pink (2200-2500 m), lightest pink (2600-2800 m) and whitish pink (>2800 m), where flower is mostly white with shade of pink colour. At different altitudes the flower also shows different pattern of spots (Nectar guides) on the corolla. Less flowering showed if trees are grown in shade as compared to those growing in open sunlight (Mamgain *et al.*, 2017) [63].

Pollination visitors

The pollinators recognized and discriminate the flower based on the specific signals such as colour, scent, size and shape. The pollination behaviours can influenced by the long distance, size of the floral display, larger flower displays generally attracting more pollination visitors (Klinkhamer and de Jong, 1990, Stout, 2000, 2007, Delmas *et al.,* 2014) [53, 111, 112, 28]. The bumblebee (Bombus) helps to pollinate the *R. semibarbatum* and *R. ponticum* (Ono *et al.,* 2008, Stout, 2007) [79, 112]. For the *R. reticulatum* and *R. macrosepalum* it was found that the flowers of both the species were visited by Hymenoptera, Lepidoptera and Diptera insects (Sugiura, 2012) [114]. Georgian *et al.,* (2015) [37] studied that the *R. floccigerum,* an ornithophious flower was pollinated by thirteen animal texa, including tow mammals and nine birds. Stevens (1976) [110] and Stevense (1985) [109] suggested that based on flower morphological features, the Papuanesia *Rhododendron* was pollinated by birds, moths and butterflies. At mt. Wilhem, the red flowered *Rhododendrons* were pollinated by birds, while white scented *Rhododendrons* pollinated by hawkmoth which supported by (Jolivet, 1998) [46]. The flowering occurs regularly twice in a year in *R. inundatum* (Argent, 2006) [4].

The *Rhododendron* and pollinator interaction was affected by some factors like flowers morphological features (Stevense, 1976, Cruttwell, 1988) [110, 26]. The white long, tubular and fragrant flower is a morphological feature of *R. inundatum* (Craven, 2007) [25]. This type of flower usually pollinated by moth mainly belongs from Sphingidae family (Stevense, 1976, Cruttwell, 1988) [110, 26]. The fragrantly white flowered *R. viscosum* produces pleasant smelt during night hours which attracts the moth pollinators (Spira, 2011) [107]. The flower of *R. indundatum* was visited by six texa of insects like Chrysopa sp. (Chrysopidae, Neuroptera), Vespidae (Hymenoptera), Curculionidae (Coleoptera), Muscidae, Drosophilidae and Tephri (Diptera). From all the insect texa the chrysopa visiting rate was highest (0.058) followed by Muscidae (0.021) and Drosophilidae (0.016) (Kuswantoro, 2017) [57]. The bees and butterflies were diurnal pollinators of white, scented *Rhododendron* flower which was mentioned by Spira (2011) [107].

Breeding behavior

In general long lived woody plant species having multiple flowering periods throughout their lifetime which shows less or low level of self-compatibility (Rambuda and Johnson, 2004) [88]. Yong-Peng *et al.,* (2014) [129] reported that the different pollination treatment including parthenogenesis test, autogamous self-fertilization test, self-pollination within a flower, geitonogamous pollination, pollinator-mediated pollination test, hand cross pollination, pollen limitation test and control applied in *Rhododendron cyanocarpum.* Out of these treatment Geitonogamous pollination showed highest fruit setting (83.30 per cent). Other treatments showed 88.60 and 86.70 per cent fruit set from hand cross pollination and pollen limitation test respectively. Based on some studies deduce a measure of inbreeding depression directly from the fruit set which are obtained by self-pollination as compared that of cross pollination (Martinez-Garcia *et al.,* 2012) [64].

A possible scenario is that the *Rhododendron cyanocarpum* is generally self-compatible, moderate amount of reproductive assurance, slightly higher fruit set in control flowers as compared to that emasculated flowers. But under the normal circumstances the out crossed progeny is fitter and under the less preventive pollinator availability the self-ed progeny is unlikely to become established (Yong-Peng *et al.,* 2014) [129]. The self-compatibility is an aspect which is an often difficult condition for pollinators in high alpine environments. This can lead to pollen limitation, which in turn may lead to such condition which can favor the self-compatibility (Escaravage and Wagner, 2004) [32].

Polyploidy

In plants evolution the central pathway is polyploidy and it is an important consideration in plant breeding as it can influence fertility, crossability, plant vigor, and gene expression (Jones *et al.,* 2007) [47]. There are more than 800 *Rhododendron* species have been recorded as diploid with $2n = 2x = 26$ (Ammal *et al.,* 1950) [1]. The *Rhododendron* chromosome number is difficult to view and count because of small in size. The chromosome numbers determination by light microscopy is therefore not a practical method for establishing ploidy levels of large numbers of individual cultivars and clones. However the Flow Cytometry is fast and accurate determinant for determination of nuclear DNA content. Flow cytometry is an effective method to detecting mixaploidy or cytochimeras, genome size and ploidy levels in Rhododendron (Jones *et al.,* 2007) [48]. The fluorescence of individual nuclei, stained with a fluorescent dye measured by the flow cytometry, while suspended in a precise stream of fluid as a means to determine genome size relative to an internal standard (Dolezel and Bartos, 2005) [29].

Moringa *et al.,* (1929) [75] the earliest cytological investigation was performed on *Rhododendron quinquefolium var*. *speciosum* N. Yonez., finding the base chromosome compliment of *Rhododendron* to be $x = 13$. Sax (1930) [94] confirmed this finding in a study that tested pollen mother cells of 16 species. Sax (1930) [94] found all the tested samples to be diploid ($2n = 2x = 26$) with the exception of two tetraploids ($2n = 4x = 52$), *R. calendulaceum* and *R. canadense* (Eeckhaut, 2004, Jones *et al.,* 2007, Li, 1957, Zhou *et al.,* 2008) [31, 47, 60, 132]. Jones *et al.,* (2007) [47] were found *Rhododendron augustinii* Hemsl. and its hybrids to be tetraploids, while *R. maddenii* Hook. f. clones to be hexaploids and octoploids. The two cultivars 'Bubblegum' and 'Northern Starburst' both were tetraploids developed from *in-vitro* colchicine treatments. Based on cytometric results they confirmed by chromosome counts on somatic cells from fifteen accessions of both *R. atlanticum* and *R. austrnum,* which showed they were tetraploids ($2n = 4x = 52$). Further investigation through cytological studies indicated that *Rhododendron canadense* may also possesses a lower base chromosome number with $2n = 2x = 24$ as compared to most of other diploid *Rhododendron* with $2n = 2x = 26$ (Lattier *et al.,* 2013) [59]. By using flow cytometry the genome size of some European and American rhododendron genotypes have been determined. On the basis of the flow cytometric data on genome size, DNA content and ploidy level of frost-resistant cultivars of rhododendrons (*R. catawbiense* 'Grandiflorum', 'Helsinki University', 'Haaga') and species (*R. adamsii*, *R. brachycarpum*, *R. dauricum*, *R. ledebourii*, *R. mucronulatum*, *R. parvifolium*, *R. schlippenbachii* and *R. sichotense*) presented (Zaytseva *et al.,* 2018) [130].

Pharmacology, phytochemistry, medicinal and other uses

Rhododendron arboreum also has an important role in pharmacology. It has been used in anti-inflammatory and anti-nociceptive activity, hepatoprotective activity, anti-diarrhoeal activity, anti-diabetic activity, antioxidants or adaptogenic activity and medicinal as well as in commercial uses (Srivastava, 2012) [108]. Besides that the different products have been produced from its bark as petroleum ether extract of the bark produces single triterpenoid substance taraxerol and ursolic acid acetate, betulinic acid and acetone extract of the bark produces leuco-pelragonidin (Hariharan and Rangaswami, 1966) [40]. The green leaves of *R. arboreum* contains glucoside, ericolin (Arbutin) ursolic acid, α-amyrin, epifriedelinol and a new triterpenoid (Campanulin), quercetin and hyperoside (Orwa, *et al.,* 2009) [80]. *R. arboreum var. nilagiricum* leaves contains hyperoside (3-D-galactoside of quercetin), ursolic acid and epifriedelinol and a triterpenoid compound (Rangaswamy and Samamurthyy, 1959) [89]. The leaves are also contains the flavones glycoside and dimethyl ester of trephthalic acid and certain flavonoides (Verma *et al.*, 2011) [123]. The flowers of *R. arboreum* contain quercetin-3-rhamnoside a crystalline chemical compound (Rangaswamy and Samamurthyy, 1960) [90]. Three biologically active phenolic compounds such as quercetin, rutin and coumaric acid have been reported from the flowers of the *R. arboreum* using high-performance thin layer chromatography (HPTLC) (Swaroop *et al.*, 2005) [116].

For the medicinal point of view *R. arboreum* dried leaves have been used for gout and rheumatism in Homeophathic Materia Medica (Skidel, 1980) [105]. "Asoka Aristha" containing *R. arboreum* possesses oxytocic, estrogenic and prostaglandin synthetase-inhibiting activity used for Ayurvedic preparation (Mildlekoop and Labadie, 1983) [68]. Dried flower of the same species are highly effective in checking of diarrhoea and blood dysentery (Laloo *et al.*, 2006) [58]. Young leaves are toxic or poisonous (intoxication in large quantity) as well as medicinal as applied it for forehead to alleviate headache (Watt, 1892) [125]. For the commercial purpose it flowers have been used for Jams, squash, jellies and local brew, acting as refreshing appetizer once using daily and prevent high altitude risk (Srivastava, 2012) [108]. Its wood has been used for charcoal and fuel and grained wood of *R. arboreum* used for making "khukri" handles, packsaddles, gift boxes, gunstocks and posts (Paul *et al.,* 2005) [83]. Traditionally the flowers of the same species have been used for offering and oblation in temples for worship and reverence as well as decoration in most of the hilly areas in Uttarakhand state of India.

Climatic hurdle

For optimal growth and flowering the species of rhododendron in general depends on physical coupled with other biological factors. Well drained, well-aerated, acidic soil rich in humus and low in elements, high rainfall, humid atmosphere, low temperature, high elevations and sun exposed slopes are optimal for bountiful growth of rhododendrons. If there is any imbalance in the ecological factors may cause deterioration of the habitat in high altitudes in rhododendrons (Bhattacharyya and Sanjappa, 2008) [10]. Rapid industrialization and high demands of society are creating a barrier in the forest growth and productivity (Mamgain *et al.*, 2017) [63]. A large number of forests are shrinking causes loss of dominant tree species due to drought and temperature induced stress (Andregg *et al.,* 2012, 2013) [2, 3]. Most often these tree species are keystone species which directly or indirectly governs the function of ecosystem. The *R. arboreum* regarded as key stone species (Mamgain *et al.,* 2017) [63]. The temperature has increased upto 1.6 ^{0}C in Himalaya (Bhutiyani *et al.*, 2007) [13] where the forests are facing risk of degradation (Pandit *et al.,* 2007, Chaturvedi *et al.*, 2011, Gopalakrishnan *et al.*, 2011) [81, 18, 39].

Due to climate change, Uttarakhand has experienced in annual mean temperature increased and rainfall decline (Mishra, 2014) [71], advancement of flowering and fruiting time (Rawat, 2013) [92], vegetation shifting upward (Bharti *et al.,* 2012, Singh *et al.*, 2012, 2013) [9, 100, 99], mean annual greenness decrease (Mishra and Chaudhuri, 2015) [72], forest degradation and fragmentation (Krishan *et al.*, 2009, Nandy *et al.*, 2011, Kaur *et al.,* 2012, Sahana *et al.*, 2016, Sharma *et al.,* 2016) [55, 78, 49, 93, 98], area decrease and fragmentation of glacier (Kumar *et al.,* 2008, Bhambri *et al.*, 2011, Bahuguna *et al.*, 2014) [56, 8, 5], early onset of spring (Bhutiyani *et al.*, 2010) [12], precocious flowering (Rawat, 2013, Ranjitkar *et al.*, 2013, Gaira *et al.*, 2014, Shah *et al.*, 2014, Singh, 2014) [92, 91, 35, 97, 103], altered leaf phenology and duration of growing season (Khanduri *et al.*, 2008, Singh *et al.*, 2010, Shreshtha *et al.*, 2012) [51, 101, 98], tree line shifting (Singh *et al.,* 2012, 2013, Schickhoff *et al.,* 2015) [100, 99, 95], plant range expansion (Schickhoff *et al.*, 2015) [95]. According to Pandit *et al.,* (2007) [81] only 10 per cent dense forest would remain in Indian Himalayan region till the year 2100.

It has been reported that the flowering is precocious in *R. arboreum* in Himalayan region because of average temperature increased (Ranjitkar *et al.*, 2013, Gaira *et al.*, 2014, Shah *et al.*, 2014, Singh, 2014) [91, 35, 96, 103]. Because due to global warming the ozone depletion and Ultraviolet rays entering in the atmosphere (Caldwell *et al.*, 2003, Paul and Gwynn-Jones, 2003, Pfeifer *et al*., 2006) [15, 84, 86] which causes early flowering in mountain plants (Ziska *et al.,* 1992) [132]. UV rays are responsible for increase production of flavonoids, which are the main photo-protective and colour imparting pigments in flower (Mamgain *et al.*, 2017) [63]. However the flowering phenology changing because of global warming which would lead to disruption of plant pollinator interaction caused extinction of pollinator ultimately decline plant population (Memmott *et al.*, 2007) [67]. Tewari *et al.*, (2016) [117] reported that the increase floral bud size positively correlated with the increase twig water potential and soil moisture.

Genome mapping and QTLs

Development of new variety with a specific trait is an achievement for all the plant species because of genetic enhancement and evolution through genome mapping and quantitative trait loci (QTLs) study. The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). Based on the phenotypic evaluation it is not possible to identify QTLs. In 1980s a major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA markers (Collard *et al.,* 2005) [22]. The process of constructing linkage maps and conducting QTL analysis to identify genomic regions which associated with traits is known as QTL mapping (also 'genetic,' 'gene' or 'genome' mapping) (McCouch and Doerge, 1995, Mohan *et al.*, 1997, Paterson, 1996 a,b) [65, 73, 82].

DNA markers like Amplified Fragment Length Polymorphism (AFPL), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs) or microsatellite, Inter-Simple Sequence Repeat (ISSR), Single Nucleotide Polymorphism (SNP) *etc.* have been used in agricultural crop species but in forest tree species limited. These markers help to construct the linkage map. Linkage maps have been utilized for identification of chromosomal regions that contain genes controlling oligogenic traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan *et al.*, 1997) [73]. DNA markers have been widely accepted as potentially valuable tools in most of the agricultural crops for improvement like rice (Mackill *et al.*, 1999, McCouch and Doerge, 1995) [61, 65], wheat (Eagles *et al.*, 2001, Koebner and Summers, 2003, Van Sanford *et al.*, 2001) [30, 54, 122], maize (Stuber *et al.*, 1999; Tuberosa *et al.*, 2003) [113, 119], barley (Thomas, 2003, Williams, 2003) [118, 127], like Tuber crops (Barone, 2004, Fregene *et al.*, 2001, Gebhardt and Valkonen, 2001) [7, 34, 36], pulses (Kelly *et al.*, 2003, Muehlbauer *et al.*, 1994, Svetleva *et al.*, 2003, Weeden *et al.*, 1994) [50, 77, 115, 126], oilseeds (Snowdon and Friedt, 2004) [106], horticultural crop species (Baird *et al.*, 1996, Mehlenbacher, 1995) [6, 66].

Many other important technologies are using in crop genome improvement in plant species such as genome editing. Current techniques of genome editing are Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and the CRISPR-Cas9 system. Which make double-stranded breaks in DNA where repairing of cells using inbuilt pathways. It is continuing search for alternative gene targeting technologies that achieved with the first reports in 2005 of zinc finger nucleases (ZFNs), and in 2010, of transcription activator-like effector nucleases (TALENs). ZFNs and TALENs are proteins that work in a conceptually in similar manner, containing one module that can be engineered to specific DNA sequence recognition and guide a second sequence where the module attached and cut the DNA (Montgomery, 2016) [74]. In 2012, it was discovered that a defence mechanism against viral attack found in the *Streptococcus pyogenes* bacterium which could be adapted as a programmable system for genome editing (Jinek *et al.*, 2012) [44]. The system comprises two elements. The first is generically termed ‘clustered regularly interspaced short palindromic repeat’ (CRISPR) RNA; the second is ‘CRISPR-associated protein 9’ (Cas9), an endonuclease (Montgomery, 2016) [74]. In 2013, the CRISPR-Cas9 system was shown to edit mammalian genomes with a high efficiency (Cong *et al.*, 2013) [23]. The genome editing mechanism can be used in *Rhododendron* species for altering the negative effect of qualitative or quantitative traits if the linkage map or genome mapping is known. Therefore, we need to identify QTLs for multi-quality traits through developing DNA markers and can be improve the genetic structure for good quality as well as adaptability.

There are many markers which can discriminate the different species of the same genus/ inter-species. However, in *Rhododendron* due to lack of genetic markers genetic studies have been obstructed. Based on RNA-seq, large-scale molecular markers were developed from four *Rhododendron* species endemic to Dabie Mountains (Central China) *viz*. *R. fortune*, *R. simsii*, *R. mariesii and R. molle.* Twelve microsatellite markers were developed from *R. simsii* which revealed that high genetic diversity in the four populations, and heterozygote excess was observed. This study would benefit to the genetic study, molecular marker-assisted selection, and breeding studies in *Rhododendron* species (Wang *et al.*, 2018) [124]. Choudhary *et al.*, (2014) [21] reported that the biotin–streptavidin hybridization technique used where 41 microsatellite markers were designed from an enriched DNA library to provide a genetic background and an insight into the population structure of the species. They reported the range 2–14 alleles amplified from 38 loci, the populations observed (0.167-.0933) and expected (0.422-0.917) heterozygosity. Some of the loci showed significant deviations from Hardy–Weinberg equilibrium and no linkage disequilibrium was detected. The used markers will support genetic diversity and further genotyping studies in *R. arboreum.*

Rhododendron protistum var. *giganteum* is the big tree rhododendron highly endangered species with only two known endemic populations in a small area of southern part of Yunnan Province in China. Unfortunately the limited information is available of this species regarding to population genetics. The result of 12 primers of AFLP found that the high genetic diversity at species level, low genetic differentiation between two populations, gene flow between populations was relatively high. Analysis of molecular variance observed the genetic variation was partitioned was 22% and 78% between population and genetic variation within population respectively. Where, 56 trees sample were clustered into two groups and concluded that this species is rare endangered and are able to maintain high genetic diversity even at a small population size. There for it assisted design of management and conservation programme (*in-situ and ex-situ*), germplasm collection for conservation and reintroduction (Wu *et al.*, 2015) [128]. Integrating the transcript and metabolite annotations produced that right from active metabolism, signaling, development, and their regulations, supplementary response to abiotic/biotic stimuli induced. It is also found that the multifaceted response displayed during flowering not only sponsored the climatic encounters but brought the shift from vegetative to reproductive growth. Overall, this comprehensive approach following transcriptome and non-targeted metabolome elucidated the contribution of genetic and metabolic factors in environmental responses (Choudhary *et al.*, 2019) [21].

Conclusion

As the nature beauty makes feel to better for each and every living organism for their entire life. The maintenance of the forest is the aims for greenness environment. The *Rhododendrons* are the flowering trees which makes adorable forest for human and animals. Due to industrialization and human interruption in forest for agriculture as well as society needs this species can be extinct in future. Where the interruption of human is a cause and other side the global warming for decline the population of such scrumptious species, conservation and protection is pledges. *Rhododendron* genus have many species but some of reached rare, keystone, endangered and near to extinct. As it produces several kind of products and paraphernalia is more important. Through traditional, scientific and molecular technologies development needs to protect the species of this genus. Otherwise most of the areas of the world will lost their identity for making the national as well as state beautiful flowers or tree. Therefore coordinate effort should be taken by government, private, NGOs policies and local community. It is necessary to save the trees, forest and environment and make the world greenness for the awesome future.

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