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# Water Stress Adaptations by *Rhododendron arboreum* Smith in the Himalayan Treeline Environment

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**Abstract.** This study examines the adaptive responses of *Rhododendron arboreum*, a key tree species in the Indian Himalayas, to water stress at the treeline ecotone (3100 - 3500 m above sea level (asl)). We measured water potential ( $\Psi$ ) using a pressure chamber and leaf conductance with an AP<sub>4</sub> type porometer. Pre-dawn water potential ( $\Psi_{PD}$ ) ranged from -0.12 to -0.91 MPa, with significant variation across sites, seasons, and years (p < 0.05). Osmotic potential at full turgor ( $\Psi\pi_{100}$ ) was -1.19 MPa for trees and -1.57 MPa for seedlings. Relative water content (RWC %) varied between 82.70 % and 91.01 %. Morning leaf conductance (gw<sub>AM</sub>) ranged from 58.00 to 271.68 mmol m<sup>-2</sup> sec<sup>-1</sup>. Soil moisture at 15 cm depth (Sm<sub>15</sub>) showed significant variation compared to deeper soil layers and leaf conductance metrics. Seedlings exhibited higher susceptibility to water stress, particularly during winter when soil freezes. Our findings highlight the eco-physiological responses of *R. arboreum* to changing environmental conditions and the impact of soil moisture dynamics on water potential and relative water content.

Keywords: Leaf conductance, osmotic potential, R. arboreum, treeline, water potential.

Classification numbers: 3.4.2, 3.5.1, 3.8.3

# **1. INTRODUCTION**

Climate has a large influence on plant recruitment [1], and at the core of regeneration, temperature and water supply are not only critical drivers for a plant's distribution [2] but also promote seed dormancy and germination. Species are assumed to occur within a certain climatic space determined by the climatic needs of the species [3]. Understanding the ecological needs of keystone species, such as *Rhododendron arboreum* [4] in the Himalayas, is of critical importance for ecosystem conservation. The *R. arboreum* occurs in the Himalayan region at elevations between approximately 1000 and 3800 m asl. Tree *rhododendrons* are vital to Himalayan ecosystems, thriving in diverse habitats like steep, high-rainfall areas with acidic soils, aiding slope stability and watershed functions [5]. Their abundant flowering from early spring to summer supports various insect and bird pollinators [6]. Alpine treeline ecotones are highly sensitive to environmental changes, particularly climate change, and can shift rapidly [7].

Drought, a critical climatic event, impacts these areas through two types: winter and summer. Winter drought, or chronic desiccation, occurs when water loss continues on sunny days but frozen soils prevent water uptake [8]. Acute frost desiccation arises from increased transpiration and reduced water absorption, often affecting species like *R. arboreum* [9]. Summer drought is driven by soil drying and high transpiration rates on warm, sunny days, further stressing plants in treeline zones.

The *R. arboreum* undergoes key phenological events in spring and summer, making its water transport system crucial for understanding its growth and survival. Studying its responses to water stress in its unique Himalayan habitat reveals adaptive strategies under extreme conditions. Key parameters like water potential, osmotic potential, and relative water content help explain how *R. arboreum* maintains water balance under varying environmental pressures. Understanding its water stress adaptations contributes to broader conservation efforts aimed at preserving biodiversity and ecosystem stability in the region because *R. arboreum* is a keystone species in the Himalayan ecosystem, providing habitat and resources for numerous other organisms. Therefore, this study seeks to explore the parameters related to water relations and adaptive strategies of *R. arboreum* trees and seedlings, especially during drier periods in the treeline areas of the Indian Himalayan region.

# 2. MATERIALS AND METHODS

# 2.1. Study sites

The present study was conducted at Aali, Bedni and Tungnath treeline in the western Himalaya at coordinates between 30°11′02″N and 79°39′36″E and altitudes between 3000 to 3500 m asl. The mean species that form the treeline were Acer caesium, Abies spectabilis, Betula utilis, Quercus semecarpifolia, Prunus cornuta, R. arboreum, R. campanulatum, and Taxus baccata.

## 2.1. Climate

The study sites are located in the alpine zone, where snow cover remains till April. Soil is acidic with pH values of 4 to 5 [10]. The climate of the study area is characterized by short cool summers and long severe winters. The mean annual temperature of the sites varied from -8.91 (January) to 25.6 °C (May) and the mean annual precipitation was  $2410.5 \pm 432.2$  mm [10].

#### 2.2. Measurements

Three soil depths were used for assessing soil moisture (Sm). At each study site, ten sample plants (trees and seedlings) were assessed for measuring  $\Psi$ , leaf conductance (gw), and water potential components of *R. arboreum* during summer, rainy, autumn, and winter seasons between 2018 and 2019.

## 2.3. Soil moisture

To estimate the level of moisture of the ground, samples of soil were taken from five representative locations at three distinct soil depths: 0-15, 15-30, and 30-45 cm. A battery-powered digital weighing balance was used to weigh 50 g of soil in the field, packaged in polybags and transported to the laboratory. The samples were dried at 100 °C until reaching a constant weight. The moisture content was then calculated using dry weight [11].

## 2.4. Water potential

A pressure chamber (PMS Instrument Co. model 1000, range 70 bars) was used to measure  $\Psi$  of trees and seedlings on each previously marked individual at pre-dawn ( $\Psi_{PD}$ ) between 5:30-6:30 hrs and mid-day ( $\Psi_{MD}$ ) between 13:30-14:30 hrs at seasonal intervals [12, 13].

# 2.5. Components of water potential

To determine the link between the components of  $\Psi$  and the relative water content (RWC %), pressure-volume (PV) curves were formed using a pressure chamber. PV curves were prepared following the bench drying method from overnight saturated twigs of trees and seedlings. The osmotic potential at zero turgor ( $\Psi\pi_0$ ), the osmotic potential at full turgor ( $\Psi\pi_{100}$ ), and the RWC % at the turgor loss point (RWCz) were all calculated from PV curves [13, 14]. Osmotic adjustment is generally calculated as the decline in osmotic potential (zero and full turgor) separately for different seasons, namely rainy, autumn, winter, and summer.

# 2.6. Leaf conductance

An AP<sub>4</sub> type porometer (Delta-T Devices) was used to measure the leaf conductance (gw) of trees and seedlings on a seasonal basis [13, 15]. The leaf conductance measurements were taken from three leaves/individuals on the sunlit sides in the morning ( $gw_{AM}$ ) between 10:30 and 11:30 and between 13:30 and 14:30.

## 2.7. Statistical analysis

Using the statistical program SPSS version 2016, the data were subjected to statistical analysis of variance and a statistically significant validation with a confidence level of 95 %. The Spearman rank correlation coefficient (r) was used to express the relationship between variables.

## **3. RESULTS AND DISCUSSION**

#### 3.1. Soil moisture

Across all the study sites and seasons the soil moisture content ( $S_m$ ) varied between 32.37 ± 1.67 and 73.50 ± 1.91 % in Year 1 (Yr1) and 31.56 ± 2.36 and 66.06 ± 1.79 % in Year 2 (Yr2). In Yr1, the maximum  $S_m$  was achieved at depths of 0-15cm during autumn while in Yr2 it reached a maximum during the rainy season and in both years, it reached a minimum at depths of 30 - 45 cm during winter (Figure 1).



Figure 1. The mean soil water content over the chosen research locations, seasons, and years. The error bars denote  $\pm$  SE.

## 3.2. Water potential of trees

 $\Psi_{PD}$  of *R. arboreum* trees ranged from -0.12 ± 0.05 to -0.91 ± 0.15 MPa in the first year (Yr1) and from -0.16 ± 0.01 to -0.88 ± 0.12 MPa in the second year (Yr2), and  $\Psi_{MD}$  of the trees was from -0.21 ± 0.03 to -1.67 ± 0.32 MPa in Yr1 and from -0.23 ± 0.03 to -1.62 ± 0.21 MPa in Yr2. Across all the study sites in both years,  $\Psi_{PD}$  was most negative in summer and  $\Psi_{MD}$  was highly adverse in the winter months and least detrimental in the rainy months (Figure 2). The ANOVA results indicated that  $\Psi_{PD}$  and  $\Psi_{MD}$  varied significantly throughout locations, years, seasons, and each interaction of  $\Psi_{PD}$  and  $\Psi_{MD}$  was also significant (p < 0.05).



Figure 2. Mean pre-dawn and mid-day  $\Psi_{\text{Tree}}$  of *R. arboreum* during all the seasons and locations. Error bars indicate  $\pm$  SE.

#### 2.1.Water potential of seedlings

 $\Psi_{PD}$  of *R. arboreum* seedlings was from -0.11 ± 0.03 to -1.27 ± 0.22 MPa in Yr1 and from -0.13 ± 0.02 to -1.0 ± 0.11 MPa in Yr2, and  $\Psi_{MD}$  of the seedlings was from -0.11 ± 0.03 to -1.91 ± 0.13 MPa in Yr1 and from -0.11 ± 0.03 to -1.76 ± 0.18 MPa in Yr2 throughout the study period and sites. Across all the study sites in both years,  $\Psi_{PD}$  and  $\Psi_{MD}$  were most negative in winter and were least detrimental in the rainy months (Figure 3). The ANOVA results showed that  $\Psi_{PD}$  of the seedlings varied significantly across years, seasons, and sites (p<0.05) and  $\Psi_{MD}$  varied significantly while the interactions of  $\Psi_{MD}$  were only significant with seasons and species (p < 0.05).



*Figure* 3. Mean pre-dawn and mid-day seedling water potential *R. arboreum* across all the seasons and sites. Error bars indicate  $\pm$  SE.

# 3.3. Daily change in water potential

The average daily variation in tree water potential ( $\Delta \Psi = \Psi_{MD} - \Psi_{PD}$ ) ranged from 0.06 ± 0.01 to 0.87 ± 0.11 MPa in Yr1, and from 0.07 ± 0.02 to 0.78 ± 0.08 MPa in Yr2, spanning all seasons and study locations. The smallest daily change in tree  $\Psi$  occurred during the rainy months, while the largest change was observed in the winter months (Figure 4). For the

seedlings, the mean daily change in  $\Psi$  was from 0.02 ± 0.01 and to 0.74 ± 0.12 MPa in Yr1 and from 0.02 ± 0.01 to 0.80 ± 0.13 MPa in Yr2, across all seasons and locations. In Yr1, the seedlings experienced the greatest daily change in  $\Psi$  during the summer season, whereas in Yr2, this peak occurred in the winter months, with the smallest changes in both years during the rainy months (Figure 4). The ANOVA results revealed that the  $\Delta\Psi$  for both trees and seedlings differed significantly across years, sites, and seasons (p < 0.05).



*Figure 4*. The daily variation in water potential for *R. arboreum* trees and seedlings across different seasons and locations. Error bars represent  $\pm$  SE.

#### 3.4. Water potential components of trees

In *R. arboreum* trees  $\Psi\pi_{100}$  was from -0.74 ± 0.01 to -1.94 ± 0.01 MPa in Yr1 and from -0.74 ± 0.01 to -1.92 ± 0.02 MPa in Yr2.  $\Psi\pi_0$  was from -0.86 ± 0.02 to -2.15 ± 0.02 MPa in Yr1 and from -0.84±0.01 to -2.18±0.02 MPa in Yr2 over the study period and locations.  $\Psi\pi_{100}$  and  $\Psi\pi_0$  were most adverse in the summer months and least adverse for both years during the rainy period (Figure 5).  $\Psi\pi_{100}$  and  $\Psi\pi_0$  declined from the rainy to the summer period.  $\Psi\pi_{100}$  declined from -0.74 to -1.94 MPa in Yr1 and from -0.74 to -1.92 MPa in Yr2 and the decline was -1.20 MPa in Yr1 and -1.18 MPa in Yr2.  $\Psi\pi_0$  declined from -0.86 to -2.15 MPa in Yr1 and from -0.84 to -2.18 MPa in Yr2 and the decline was -1.29 MPa in Yr1 and -1.34 MPa in Yr2 (Figure 5).

Across all seasons and locations, the pressure potential at full turgor ( $\Psi$ p) varied from 0.57 ± 0.02 to 1.56 ± 0.01 MPa in Yr1 and from 0.56 ± 0.01 to 1.49 ± 0.08 MPa in Yr2. For both years,  $\Psi$ p peaked in summer and decreased in the rainy season (Figure 5). The RWC% was from 84.12 ± 0.99 to 90.76 ± 0.45 % in Yr1 and was from 82.70 ± 0.78 to 91.01 ± 0.41 % in Yr2, throughout all the study seasons and locations. RWC% peaked in summer of Yr1 and winter of Yr2, while it was lowest in the rainy period in both years (Figure 5). The results of ANOVA showed that there were significant differences (p < 0.05) in  $\Psi\pi_{100}$ ,  $\Psi\pi_0$ , PP<sub>full</sub>, and RWC% in all seasons and sites, but not in the two research years. All other interactions were not significant, except for the interaction between seasons and sites, which varied significantly (p < 0.05).



*Figure 5*.  $\Psi \pi_0$ ,  $\Psi \pi_{100}$ ,  $\Psi p$  and RWC% of *R. arboreum* trees across various seasons and locations. Error bars denote  $\pm$  SE.

## 3.5. Water potential components of seedlings

In *R. arboreum* seedlings  $\Psi \pi_{100}$  was from -0.72 ± 0.16 to -2.14 ± 0.03 MPa in Yr1 and from -0.77 ± 0.05 to -2.49 ± 0.06 MPa in Yr2.  $\Psi \pi_0$  ranged from -1.63 ± 0.39 to -4.20 ± 0.24 MPa in Yr1 and -1.79 ± 0.26 to -3.67 ± 0.11 MPa in Yr2 throughout the study period and locations. The  $\Psi \pi_{100}$  and  $\Psi \pi_0$  was highly negative in summer for both years while  $\Psi \pi_{100}$  was minimal adverse in the rainy period and  $\Psi \pi_0$  was minimal adverse in autumn during the study period (Figure 6).  $\Psi \pi_{100}$  declined from rainy to the summer season and  $\Psi \pi_0$  declined from autumn to summer months during the study period.  $\Psi \pi_{100}$  declined from -0.72 to -2.14 MPa in Yr1 and from -0.77 to -2.49 MPa in Yr2 and the decline was -1.42 MPa in Yr1 and -1.72 MPa in Yr2.  $\Psi \pi_0$  declined from -2.63 to -4.20 MPa in Yr1 and from -1.79 to -3.67 MPa in Yr2 and the decline was -2.57 MPa in Yr1 and -1.88 MPa in Yr2 (Figure 6).

Ψp in *R. arboreum* seedlings ranged from  $1.25 \pm 0.11$  to  $1.94 \pm 0.11$  MPa in Yr1 and  $1.14 \pm 0.09$  to  $2.06 \pm 0.12$  MPa in Yr2 over the study periods and locations. Ψp was highest in the winter months and lowest during the rainy months over the study period (Figure 6). Across all the seasons the RWC% varied from  $54.20 \pm 3.96$  to  $87.80 \pm 2.80$  % in Yr1 and  $56.60 \pm 2.83$  to  $85.00 \pm 3.13$  % in Yr2. RWC% reached a minimum in the summer season and a maximum in the winter months throughout the study (Figure 6). The ANOVA results revealed that  $\Psi \pi_0$ , PP<sub>full</sub>, and RWC% of *R. arboreum* seedlings varied significantly across sites and seasons (p<0.05), but not in two study years.  $\Psi \pi_{100}$  varied significantly across locations (p<0.05) but not significantly by study years and seasons. All other interactions were not significant, except for the one between seasons and locations, which varied significantly (p < 0.05).



*Figure 6*.  $\Psi \pi_{0}$ ,  $\Psi \pi_{100}$ ,  $\Psi p$  and RWC% of *R. arboreum* seedlings across various seasons and locations. Error bars denote  $\pm$  SE.

# 3.6. Leaf conductance of trees

 $gw_{AM}$  values of the *R. arboreum* tree ranged from  $58.00 \pm 2.50$  to  $245.08 \pm 3.87$  mmol m<sup>-2</sup> sec<sup>-1</sup> in Yr1 and from  $63.24 \pm 2.11$  to  $271.68 \pm 4.09$  mmol m<sup>-2</sup> sec<sup>-1</sup> in Yr2, and  $gw_{PM}$  was from  $44.64 \pm 3.00$  to  $163.78 \pm 4.02$  mmol m<sup>-2</sup> sec<sup>-1</sup> in Yr1 and from  $95.68 \pm 2.95$  to  $411.52 \pm 5.17$  mmol m<sup>-2</sup> sec<sup>-1</sup> in Yr2 over the study locations and period. During the study years,  $gw_{AM}$  reached a minimum in the summer months and a maximum in the rainy months, and  $gw_{PM}$  was minimal during the winter period in Yr1 and the rainy period in Yr2 (Figure 7). ANOVA revealed that the forenoon and afternoon gw of *R. arboreum* varied significantly with locations, years and seasons (p < 0.05). All morning leaves conductance interaction was also significant (p < 0.05).



*Figure* 7. Mean leaf conductance (mmol  $\text{m}^{-2} \sec^{-1}$ ) of *R. arboreum* trees in the morning and afternoon during all study seasons, locations, and years. The error bars show  $\pm$  SE.

#### 3.7. Leaf conductance of seedlings

The morning leaf conductance of *R. arboreum* seedlings was from  $46.00 \pm 2.14$  to  $589.00 \pm 21.67 \text{ mmol m}^{-2} \text{ sec}^{-1}$  in Yr1 and from  $47.20 \pm 2.09$  to  $460.00 \pm 18.89 \text{ mmol m}^{-2} \text{ sec}^{-1}$  in Yr2. Similarly, the leaf conductance during the afternoon was from  $24.00 \pm 1.36$  to  $312.00 \pm 19.82 \text{ mmol m}^{-2} \text{ sec}^{-1}$  in Yr1 and from  $26.00 \pm 2.89$  to  $300.98 \pm 22.27 \text{ mmol m}^{-2} \text{ sec}^{-1}$  in Yr2. Both the gw<sub>AM</sub> and gw<sub>PM</sub> were highest for the rainy months and lowest for the winter months over the study years, locations, and periods (Figure 8). The ANOVA results indicated that the morning and afternoon gw<sub>seedling</sub> of *R. arboreum* varied significantly with years, seasons, and sites (p < 0.05). All the interactions also varied significantly with each other (p < 0.05).



*Figure 8.* Mean leaf conductance (mmol m<sup>-2</sup> sec<sup>-1</sup>) of *R. arboreum* seedlings in the morning and afternoon during all study seasons, locations, and years. The error bars show  $\pm$  SE.

## 3.8. Relationships between the variables

The relationship between several factors revealed that the soil moisture,  $Sm_{15}$  varied significantly with  $Sm_{30}$ ,  $Sm_{45}$ ,  $gw_{AM}$  and  $gw_{PM}$  of the seedlings;  $Sm_{30}$  varied significantly with  $Sm_{45}$ ,  $gw_{AM}$  and  $gw_{PM}$  of the seedlings;  $Sm_{45}$  only varied significantly with  $gw_{AM}$  of the seedlings;  $\Psi_{PD}$ ,  $\Psi_{MD}$ ,  $gw_{AM}$  and  $gw_{PM}$  of the trees and seedlings varied significantly with each other at a significant level of 5 % (p < 0.05) (Table 1). A positive correlation was observed between morning and afternoon tree and seedling leaf conductance with pre-dawn water potential. The leaf conductance rate of *R. arboreum* seedlings increases, resulting in an increase in water potential. In trees the maximum morning and afternoon leaf conductance was measured when the water potential was from -0.50 to -0.90 MPa (Figure 9).

Variable	Sm <sub>15</sub>	Sm <sub>30</sub>	Sm <sub>45</sub>	Tree				Seedling			
				$\Psi_{PD}$	$\Psi_{MD}$	gw <sub>AM</sub>	gw <sub>PM</sub>	$\Psi_{PD}$	$\Psi_{MD}$	gw <sub>AM</sub>	gw <sub>PM</sub>
Sm <sub>15</sub>	1	0.95**	$0.84^{**}$	$-0.23^{NS}$	$-0.20^{NS}$	-0.41 <sup>NS</sup>	$-0.06^{NS}$	-0.39 <sup>NS</sup>	$-0.40^{NS}$	-0.66**	-0.59**
Sm <sub>30</sub>		1	0.94**	$-0.22^{NS}$	-0.11 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.27 <sup>NS</sup>	-0.31 <sup>NS</sup>	-0.59**	-0.49**
Sm <sub>45</sub>			1	-0.11 <sup>NS</sup>	0.01 <sup>NS</sup>	0.13 <sup>NS</sup>	-0.05 <sup>NS</sup>	0.17 <sup>NS</sup>	-0.19 <sup>NS</sup>	-0.50**	-0.37 <sup>NS</sup>
$\Psi_{PD}$				1	0.91**	$0.77^{**}$	$0.47^{**}$	1	0.93**	0.81**	$0.89^{**}$
$\Psi_{MD}$					1	$0.67^{**}$	$0.48^{**}$		1	$0.77^{**}$	0.91**
gw <sub>AM</sub>						1	$0.68^{**}$			1	$0.90^{**}$
gw <sub>PM</sub>							1				1

Table 1. Spearman rank correlation coefficients between various R. arboreum water relations variables.

Note: NS = Correlation is non-significant, \*\* = Correlation is significant at the 5 % level (p<0.05) (2-tailed).



*Figure 9.* Relationships between forenoon and afternoon leaf conductance with pre-dawn water potential of *R. arboreum* trees and seedlings.

The severity of drought can be assessed by the minimum level of  $\Psi$ , with the most severe site mean of  $\Psi_{PD}$  serving as a key indicator [16]. At higher elevations, increased water vapor reduces the impact of solar radiation and elevates the importance of low temperatures, affecting water availability to plants [17]. In this study, the high soil moisture content across all treeline sites can be attributed to substantial rainfall and heavy snowfall spanning about 8 to 9 months.  $\Psi_{PD}$  and  $\Psi_{MD}$  of *R. arboreum* trees remained above -0.91 and -1.67 MPa, respectively, while for the seedlings, they remained above -1.27 and -1.92 MPa, suggesting the absence of severe stress in both trees and seedlings in these treeline areas. This absence of severe stress could be due to snowmelt from winter snowfall maintaining soil moisture during spring and summer seasons before monsoon rains, as observed in Himalayan treeline areas [13]. Studies on R. arboreum and *R. campanulatum*, revealed  $\Psi_{PD}$  above -1.0 MPa [18]. However, these species, characterized by small vessel diameters, are sensitive to drought-induced embolism [19]. Notably, the most negative  $\Psi_{PD}$  and  $\Psi_{MD}$  of seedlings and  $\Psi_{MD}$  of trees were observed during winter. In treeline species, water supply issues can arise during winter months when the ground freezes, impeding water uptake crucial for foliar maturation and leading to winter desiccation in young individuals lacking snow protection above the treeline [20]. Seedlings are particularly susceptible to highstress levels as they depend on water in shallow soil layers where moisture availability is rapidly depleted [21]. Comparing  $\Psi_{PD}$  of the sub-alpine (3000-3300 m) R. arboreum with the subtropical (1500 - 1800 m) R. arboreum of the Indian Himalayan region (IHR),  $\Psi_{PD}$  of the subalpine R. arboreum was approximately half that of the R. arboreum measured at sub-tropical regions [16, 22 - 24]. This suggests that the R. arboreum species in sub-tropical and temperate regions face relatively more severe water deficits than those growing in treeline areas.

Examining daily fluctuations in water potential provides crucial insights into a plant's overall water status. In our study,  $\Delta \Psi$  ranged from 0.06 to 0.87 MPa in trees and from 0.02 to

0.80 MPa in seedlings. These variations in plant  $\Psi$  observed both from day to day and at different times within the same day, were influenced by a combination of environmental conditions and physiological processes. Notably, higher variations in  $\Delta\Psi$  were often linked to increased transpiration rates, more negative leaf  $\Psi$ , and elevated photosynthesis levels. However, in treeline areas,  $\Delta\Psi$  of *R. arboreum* trees and seedlings appeared to be comparatively low. The low  $\Delta\Psi$  might be attributed to the prevailing climatic conditions such as abundant rainfall, low temperatures, high soil moisture, and humidity levels.

As drought conditions worsen, cells respond by adjusting their internal water balance through the accumulation of osmotic solutes. In R. arboreum, exhibited a decline in osmotic potential from the rainy season to summer, at both full and zero turgors. Similar trends were reported by previous studies in comparable treeline areas [13, 25]. This pronounced reduction in osmotic potential suggests that the primary strategy of R. arboreum is to ensure water availability, particularly to maintain high rates of photosynthesis during warm, sunny days with optimal humidity [13]. Osmotic adjustment is evident when  $\Psi \pi_{100}$  declines [26]. In *R. arboreum*, seasonal changes in  $\Psi \pi_{100}$  were observed, with mean values of -1.19 MPa in trees and -1.57 MPa in seedlings. This notable decline in osmotic potential during mild drought conditions should facilitate the retention of soil water, thereby supporting fall photosynthesis [26]. Species with deep roots or efficient water transport mechanisms often exhibit less osmotic adjustment compared to shallow-rooted trees [27]. Relative water content at turgor loss point (RWCz) remained relatively high during both winter and summer seasons, coinciding with the period of most negative  $\Psi$ . Moreover, *R. arboreum* maintained a high RWCz ranging from 89.92 % to 91.01 % during peak drought conditions. The ability of trees to uphold high RWC under drought stress was also observed in other studies [28, 29].

Plants regulate water loss by controlling stomatal aperture, influenced by the water potential gradient between the leaf and surrounding air [30]. In this study, gw was highest during the rainy season and lowest from mid-September to mid-February. High humidity and low vapor pressure deficit during the rainy season promoted optimal stomatal conductance. Stomatal responses are interconnected with soil moisture, leaf  $\Psi$ , and atmospheric conditions, with reduced conductance during dry periods correlating inversely with  $\Psi_{PD}$  in oak trees [31]. Similarly, it was also observed that the stomatal conductance decreased in dry months when tree water potential was at its lowest [15]. The rate of stomatal response to water deficits affects transpiration, photosynthesis [32], carbon uptake, and other physiological processes within plants. During diurnal measurements in the winter season, leaf  $\Psi$  corresponded with gradual increases in leaf stomatal conductance and vice versa, likely because plants received a steady water supply throughout the day [33].

## 4. CONCLUSIONS

This study emphasizes the importance of conserving Himalayan ecosystems, particularly *R*. *arboreum* and other treeline species. Sustainable water management, like rainwater harvesting and soil moisture conservation, is crucial to address seasonal water variability. Conservation efforts should be tailored to the species' specific responses to water stress and integrate climate resilience, while also involving local communities. As a keystone species, *R. arboreum* maintains ecological balance, but winter soil freezing threatens water uptake, especially for seedlings. Long-term research on water potential and ecological factors will deepen understanding of the species' water dynamics.

*CRediT authorship contribution statement.* Nandan Singh: Conceptualization, Methodology, Data collection, Data analysis, Writing original draft, Editing and Finalization. Ashish Tewari: Conceptualization, Methodology, Data collection, Visualization, Supervision, Review and Finalization. Shruti Shah: Conceptualization, Methodology and Data collection. Amit Mittal: Conceptualization, Methodology and Data collection, etc.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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