

Assessment of growth, floral and biochemical characteristics of wild rose (*Rosa* **sp.) across varied elevations of Rawalakot, Azad Jammu and Kashmir**

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Abstract

Roses have held their status as exquisite decorative plants since ancient times, captivating people with their timeless beauty. The rising demand for roses as an industrial raw material has led to a significant increase in their value. The picturesque state of Azad Jammu and Kashmir is home to flourishing large-scale rose plantations, particularly in the stunning wilderness of Rawalakot, located in District Poonch. The objective of this study was to collect wild roses from four distinct locations, each characterized by a unique elevation, and to evaluate their growth, floral, and biochemical characteristics. These locations include Khai Gala at 6329 ft, Chota Gala at 5744 ft, Mutyal Mehra at 5341 ft and Drake at an elevation of 3378 ft. The comparative analysis of the roses revealed that those cultivated at higher elevations exhibited a remarkable increase in growth characteristics and petal diameter, with measurements indicating a substantial difference from their counterparts grown at lower elevations. Furthermore, the analysis of rosehip content demonstrated a significantly higher concentration of essential nutrients (such as total soluble solids: 40% ; vitamin C $49.8 \text{ mg}/100 \text{ gFW}$; total flavonoids 1.73 mg/100 g FW) in the rosehips of the higher-altitude roses, making them a more nutritionally dense source. The findings from the study demonstrate that the specific environmental conditions and variations in elevation significantly influence the physical characteristics and biochemical composition of wild roses. This suggests that there are opportunities to enhance the quality of roses through the implementation of tailored cultivation methods designed to accommodate the diverse elevations at which they grow.

Keywords: Altitudes, Biochemical parameters, Morphological variations, Wild rose

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Introduction

Rosaceae is the family that includes the wild rose (*Rosa* sp.) (Roberts, 2003). Rose (*Rosa indica*) is highly significant commercial flower crops that is commonly known as the "queen of flowers" (Hegde, Gupta, Sharma, Srivatsan, & Kumari, 2022). This plant, characterized by its sturdy, hardy woody stem and adorned with sharp, pointed thorns with more than hundred species and 25000 cultivars and are native from Asia while some species are native from North America, Africa and Europe (Belal et al., 2016). During both times of peace and war, roses are universally recognized as symbols of beauty and love (Farooq et al., 2013). A significant increase in rose production was observed in the early 20th century following the Second World War (Monder, 2021). Currently, the Chinese pharmacopeia dates back 4,000 years, using rose petals, buds and hips as a tea. A common Indian traditional remedy for cough and throat infections is made from processed rose petals, known as *Gulkand* (Hegde et al., 2022). Roman, Egyptian, and other ancient and prehistoric societies all used roses as a decorative element (Radanova, 2023).

 Roses are very popular for landscaping and as cut flowers because of their extraordinary aroma and beauty. With more than a hundred species, this genus is extensively dispersed in Europe, North America, Asia, and the Middle East (Basu et al., 2014). Furthermore, roses are extremely beneficial to human health. With 28,130 hectares under cultivation, India leads the globe in rose production. China comes in second with 14,316 hectares and Ecuador with 4,073 hectares (Basu et al., 2014). The Netherlands, with 775.5 million euros, Kenya, with 299.5 million euros, and Ethiopia, with 136.3 million euros, are the top exporters. It's interesting to note that, with imports totaling 416.3 million euros, the Netherlands is also the world's biggest rose importer, demonstrating its supremacy in the world rose market. Pakistan has the most fertile soils and suitable temperature that is ideal for flowers, therefore roses have been

grown there for a few decades. Roses may grow in many different types of climates in Pakistan, including hot, cold, dry, and humid ones. Even with this flexibility, the amount of land used for fresh-cut rose production is still quite small just 1,500 acres (Farooq et al., 2013). This acreage stakes in comparison to other cash crops cultivated in the country. Many of the flowers grown in Pakistan are wasted, while a limited proportion is exported to the Middle East and used locally (Almas et al., 2023). According to research, one of the main reasons for smallscale flower farming in agriculture is a lack of knowledge about the practice. They also found that the country's climate is ideal for flower growing(Leghari et al., 2015).

 There are about 25 species of Rosa known to grow wild, and many variants have improved the modern ornamental and beautiful rose. Notably, it has been noted that a number of rose species are spreading quickly over northern areas of Pakistan. These roses grow well in a variety of conditions, from dry locations to temperate climates, demonstrating their endurance and flexibility (Tabaei-Aghdaei et al., 2007). All other rose subspecies, with the exception of few that are primarily found at high elevations, flourish at mid- to low elevations (Lewis & Ertter, 2007). The rose can be found in a variety of settings due to its wide range of environmental adaptations. It can be found as an underwood plant in both dry and moist, shaded environments (Rossman, 2017). In Pakistan, especially in the northern areas and Azad Jammu and Kashmir, there are several wild species of Rosa. Enhancing these species using conventional breeding methods or contemporary molecular techniques may result in large financial gains for the community (Abdul Aziz & Masmoudi, 2024). This is especially important in places where conventional agriculture is the primary source of income for farmers, who usually possess less than one hectare of land (Aćin et al., 2023). Therefore, the present study was designed to collect samples of wild roses from various locations including lower and higher altitudes. After collection of samples, different growth, floral and biochemical characteristics were noted.

Materials and Methods

Description of the field site

Wild rose plants were collected from four different locations including Khai Gala (Latitude: 33°50'53"N, Longitude: 73°49'25.62"E, Elevation: 6329 ft), Chota Gala (Latitude: 33°49'45"N, Longitude: 73°48'33"E, Elevation: 5744 ft), Mutyal Mehra (Latitude: 33°51'31" N, Longitude: 73°45'35" E, Elevation: 5341 ft), Drake (Latitude: 73°77'81" N, Longitude: 33°82'40" E, Elevation: 3378 ft). From each location, ten plants were chosen. After that, from each plant five branches from the north, south, east, west, and middle were tagged. Upon blossom maturity, three flowers were removed from each branch and brought to the Laboratory of Department of Horticulture, University of Poonch Rawalakot for recording different parameters.

Growth characteristics

Numerous metrics were used in this study to describe the plant's growth and development.

Plant height

To measure the plant height (cm) (from the top to the bottom of main stem), measuring tape was used.

Plant canopy

The entire area that the plant's top covered from one end to the other was measured in centimeters to determine the plant canopy $(cm²)$.

Number of primary and secondary branches

Number of primary and secondary branches per plant was counted manually and their average was taken.

Number of leaves

Number of leaves per plant was counted manually and their average was taken.

Number of thorns

Number of thorns per plant was counted manually and their average was taken.

Size of thorn

A measuring tape was used to determine the thorns average size in cm.

Floral characteristics

Numerous floral features were studied:

Number of flowers

Flowers on each plant were counted manually.

Number of petals per flower

Number of petals per flower were counted and their average was taken.

Size of petal per flower

Using a measuring tape, the average petal size per flower was calculated in centimeters.

Rosehip diameter

Rosehip weight (g) was calculated with a digital scale.

Rosehip weight

Rosehip diameter (mm²) was measured with Vernier calipers.

Biochemical characteristics

pH

pH of petals was assessed by pH meter (Model: WTW 82362 Inolab, Germany). pH meter was adjusted with pH buffers 4.0, 9.0 before readings.

Total soluble solids (%)

A hand refractometer (Kyoto Company, Japan) was used for total soluble solids. A single drop of petal juice was placed on the dry refractometer prism. The data was then recorded in percent $(\%).$

Vitamin C (mg/100)

The AOAC method was used to determine the amount of vitamin C in each sample. Using a 2, 6-dichlorophenol indophenol dye, vitamin C was measured. A conical flask filled with 100 ml of 4% Meta phosphoric acid solution plus 5 ml of the extracted juice. A 2, 6-dichlorophenol indophenol dye was added and titrated until a bright pink color was reached.

Total flavonoids (mg/100 g FW)

The flower (0.5 mg) was crushed into a consistent paste (0.5 mg) using a mortar and pestle. Afterwards1.5 ml of methanolic $AICI_3.6H_2O$ was mixed with the homogenized flower in sealed tubes, and the combination was left in the dark for fifteen minutes. By using a UV-4000 spectrophotometer (UV-4000, Hamburg, Germany), the absorbance was determined at 430 nm. The results were provided in milligrams per 100 grams of fresh weight (mg/100 g FW) (Ali, Zahid, Manickam, Siddiqui, & Alderson, 2014).

Total phenols (µg of Gallic acid per FW)

The total phenolic content was determined using spectrophotometric analysis. A 0.5 ml of folin-Ciocalteau is added to 1.5 ml of sodium carbonate and 0.1 ml of homogenized petal juice. To measure the absorbance at 750 nm, we used an ultraviolet spectrophotometer (UV-4000, Hamburg, Germany). The fresh weight of the fruit sample was expressed as μ g of GA for each result.

Total anthocyanins (mg of cyaniding-3-glucoside per liter)

Total anthocyanins were calculated using two different dilution procedures (Zheng, Wang, Wang, & Zheng, 2007). Results were expressed in mg of cyanidin-3-glucoside/l.

Total antioxidant activity

FRAP assay was used to measure antioxidant activity according to the method given by (Zahid et al., 2022). For this analysis, 40 µl of rose petal extract was homogenized with 3 ml of FRAP reagent. The resulting mixture was then incubated for 4 minutes in a dark.

Statistical analysis

The data was carefully examined and analyzed using descriptive statistics with the use of Statistix 8.1. Following this, an analysis of variance (ANOVA) was done to compare the means. At a 95% confidence level (P≤0.05), we used the Least Significant Difference test to differentiate the mean values among the four locations.

Results

Growth characteristics

Evidence about growth traits of wild roses at various altitudes was gathered and statistically (P≤0.05) examined between altitudes (Table 1). Maximum plant height was observed at Khai Gala (167.57 cm) while the lowest plant height was recorded at Drake (145.77 cm). Maximum plant canopy was measured at Khai Gala (172.97 cm^2) while the lowest plant canopy was recorded at Drake (135.07 cm^2) (Table 1). Data was collected and analyzed using statistical methods to document the varying counts of primary branches, secondary branches and leaves per plant across different altitudes. Significant differences (*P*≤0.05) among elevations were observed (Table 1). Maximum number of primary branches per plant (19.27), maximum number of secondary branches per plant (13.46), maximum number of leaves per branch (72.60) was observed at highest location (Khai Gala) (Table 1). Data regarding number of thorns per plant showed maximum number of thorns (61.86) at lowest location (Drake) whereas, minimum number of thorns (61.86) was recorded at highest location (Khai Gala) (Table 1). Data regarding size of thorns showed large sized thorns (1.713 cm) at lowest location (Drake) whereas, small sized thorns (1.133 cm) was recorded at highest location (Khai Gala) (Table 1).

Floral characteristics

Data regarding floral characteristics of wild rose at different altitudes was recorded and analyzed statistically (*P*≤0.05) among different altitudes (Table 2). Maximum number of flowers per plant was observed at Khai Gala (17.65) while the minimum plant height was recorded at Drake (12.64).

Maximum number of petals per flower was measured at Khai Gala (14.27) while the minimum number of petal per flower was recorded at Drake (12.64) (Table 2). Data regarding size of petals per flower showed maximum size (1.45 cm^2) at highest location (Khai Gala) whereas, minimum size of petals per flower (1.10 cm^2) was recorded at lowest location (Drake) (Table 2). Data regarding rosehip diameter and rosehip weight showed maximum rosehip diameter (2.51 mm^2) and maximum rosehip weight (0.57 g) at highest location (Khai Gala) whereas, minimum rosehip diameter (1.80 mm^2) and minimum rosehip weight (0.51 g) was recorded at lowest location (Drake) (Table 2).

Biochemical characteristics

The roses that were collected from various locations revealed substantial (P≤0.05) differences in pH, total soluble solids, and vitamin C (Fig. 1). The pH results for roses grown at various elevations revealed a considerable (P≤0.05) variation (Fig. 1a). Our findings showed that the lowest pH (Fig. 1a) was observed at lower height and the greatest pH (3.78) at higher elevation. Total soluble solids of roses grown at four different elevations showed a significant ($P \le 0.05$) difference (Fig. 1b). At the greatest altitude (Khai Gala), the total soluble solids value was at its highest (4.0%). At the lowest altitude, however, only 2.8% of the total soluble solids were measured (Fig. 1b). When vitamin C (mg/100g FW) was cultivated at four different heights, a significant (P≤0.05) difference was seen (Fig. 1c). At the greatest altitude (Khai Gala), vitamin C levels were at their highest $(49.8 \text{ mg}/100 \text{ g} \text{FW})$. A low level of vitamin C (36.9 mg/100 g FW) was observed in roses grown at the lowest altitude (Fig. 1c). The total flavonoids, total phenols, and total anthocyanin results for the roses gathered from various elevations revealed a

significant (P≤0.05) variation (Fig. 2). The total flavonoid results revealed that roses grown at different elevations differed significantly (P≤0.05) (Fig. 2a). According to our findings, higher elevations were associated with the highest total flavonoids (1.73 mg/100 g FW), whereas lower elevations were associated with the lowest total flavonoids (1.29 mg/100g FW) (Fig. 2a). Total phenols of roses grown at four different elevations showed a significant ($P \le 0.05$) difference (Fig. 2b). At the greatest altitude (Khai Gala), the total phenol content was at its maximum amount (3.41 mg/100 g FW). Lowest altitude (2.76%) had the lowest percentage of total phenols observed (Fig. 2b). A significant ($P \le 0.05$) difference was observed in total anthocyanin (mg of cyanidine-3 glucoside/100 g FW) grown at four different elevations (Fig. 2c). Highest value (3.90 mg of cyanidine-3-glucoside/100g FW) of total anthocyanin was recorded at highest altitude (Khai Gala). While low value (2.5 mg of cyanidine-3 glucoside/100g FW) of total anthocyanin were recorded in rose grown at lowest altitude (Fig. 2c). A significant ($P \le 0.05$) difference was observed in total antioxidants activity $(\mu g/100g)$ FW) grown at four different elevations (Fig. 2d). Highest value (3.36 µg /100 g FW) of total antioxidants activity was recorded at highest altitude (Khai Gala). While low value (1.65 µg/100 g FW) of total antioxidants activity were recorded in rose grown at lowest altitude (Fig. 2d). Table 3 indicates the positive correlation among the biochemical parameters under study. Table 3 showed that 1 degree increase in total soluble solids results in 0.4348 times increase in vitamin C and vice versa. Similarly, 1 degree increase in vitamin C results in 0.9195 times increase in total flavonoids. With 1 degree increase in total flavonoids, the amount of total phenols increases to 0.4354 times. All the values showed that total antioxidants are positively correlated with all the other biochemical characteristics under study.

Location	Plant	Plant canopy	No. of primary	No. of	No. of	No. of	Size of
	height	cm^2)	branches/plant	secondary	leaves/plant	thorns/plant	thorn (cm)
	cm			branches/plant			
Khai Gala	167.57 a	172.97 a	19.27 a	13.46 a	72.60a	61.86 d	1.133 d
Chota Gala	164.33 b	163.07 b	16.86 _b	12.66a	67.27 b	67.16c	1.230c
Mutyal	152.90 c	146.70c	15.53 bc	11.10 _b	56.30 c	75.00 b	1.316c
Mehra							
Drake	145.77 d	135.07 d	13.70c	10.26 _b	53.16 d	81.36 a	1.713a
C.V	3.23	2.33	2.32	1.08	2.68	3.42	0.029

Table 1 Growth characteristics of wild roses collected from four different locations of Rawalakot

Means with different letters are significantly different (*P*≤0.05) from each other in each column

Means with different letters are significantly different (*P*≤0.05) from each other in each column

All the values are significantly correlated (*P*≤0.05).

Discussion

Rose is cultivated widely around the world but the location and cost of production depends upon the logistic aspects, labor cost and environmental factors (Cola et al., 2020). The goal of the present study was to assess various morphological and biochemical parameters and their effects on the performance of wild roses across four different locations. The height of wild roses is inclined by environmental conditions such as sunlight, elevation, high temperature and wind speed (Zeynali et al., 2009). These results are consistent with our research, which showed that higher altitudes had more sun light which resulted in high temperatures (Zahid et al., 2021). All the physiological factors such as plant height, canopy, primary and secondary branches, number of flowers, number of petal and rosehip diameter are highly dependent on temperature (Buwalda et al., 1999). This high temperature may lead to variation in lipid membranes which enable in accumulation of certain proteins that decreases water contents and helps in overcoming all the abiotic stresses which resultantly helps in increased developmental and floral characteristics (Kose & Kaya, 2022). In another study it was noticed that high number of carpels and petals were produced at high day temperature and low night temperature (Yeon et al., 2022). In our results, Khai Gala is situated at higher altitude maintained high day temperature and low night temperature.

 Number of branches and number of leaves per plant varied with the variations in elevations, ecological stress, genetic composition (Nybom et al., 1997). More number of leaves per plant were observed at higher elevations which could be due to more decomposed organic matter at higher elevations (Zahid et al., 2021) which helped in maximum plant growth (Ziogou et al., 2023). Changes in altitude may have a great impact on morphological characteristics of plants such as leaf length, branch count, plant height, canopy and thorn size, which could be due to more sunlight, higher temperature and better soil fertility. In our results Khai Gala showed significant increase in developmental characteristics of wild roses. This might be due to very low night temperature, as low temperature helps in production of different enzymes such as sucrosephosphate synthase stromal fructose-1, 6-bisphosphatase and Rubisco (Yamori et al., 2005). All these enzymes play a crucial role in augmenting photosynthesis rate, thereby stimulating vigorous plant growth.

 In our results flowers at higher elevations had larger petals in comparison to lower elevations, which could be due to more sunlight at higher elevations (Farooq et al., 2013). More sunlight results in more photosynthesis which in turns results in increased number of petals with improved diameter. Similar results were obtained for rosehip diameters (Demir & Özcan, 2001). The weight, shape, and diameter of fruits are among the characteristics that are impacted by elevation (Soare et al., 2015). Roses grow best between 20-25 °C during daytime, with 8 hours of sunlight and relative humidity $\leq 75\%$ coupled with 1316 °C during night time. Although roses can be cultivated at temperatures less than 15 °C but this results in longer time duration for flower bloom with flat topped buds which reduces flower quality. This longer timer for flower bloom is might be due to the fact that some physiological problems produced by temperature below 5 °C. Conversely, roses grown at temperatures higher than 30 °C results in poor flower quality but fewer petals. Many roses are grown in tropical places, such as Kenya and Ecuador with average temperature between 15-20 °C. These areas produces high quality roses that might be imported to other countries (Desta et al., 2022). In our results the plants grown at higher elevations are has the maximum number of leaves and flowers with higher nutritional quality. The higher elevation maintained the suitable temperature for rose yields and quality (Desta et al., 2022).

 The pH fluctuations were ascribed to factors such as elevation, climate, and titratable acidity (Scalzo et al., 2005; Mabellini et al., 2011). The vitamin C content of wild rosehip was found to vary depending on species, location and weather. However, it was shown to be four times more than that of blackcurrants and 30-40 times more than oranges in the same quantity (Ghazghazi et al., 2010; Rosu et al., 2011). Rosehip is known as a natural reservoir for valuable flavonoids. In our results, the change in flavonoids content indicates that flavonoids and antioxidants activity is genotype-specific which varied in rosehips with the variation in elevation level (Adamczak et al., 2012; Roman et al., 2013; Murathan et al., 2016). These results revealed that the nutritional composition of rosehip is highly versatile which contains anthocyanins, vitamin C and flavonoids, while genotype plays major role. In addition to vitamin C, tocophenol and pigments some other bioactive substances are found in wild roses, and all these bioactive substances interact with each other and are responsible to scavenge free radicals (Wang et al., 2023). Vitamin C is also known as ascorbic acid and composed of two adjacent enol hydroxyl groups at second and third position of its molecule, and this hydroxyl group easily dissociate and release H^+ . This H^+ ions gives acidic characteristics to vitamin C Various studies revealed that concentration of vitamin C varies with growth processes, variety, maturity and altitude (Chen et al., 2021). Oxygen level decreases at higher altitude which reduces the oxidative stress in the plants. Low temperature and high altitudes causes a reduction in respiratory rate of rosehip which results in lower oxygen contents in fruits. Consequently, the reduction in oxygen content in fruit reduces plants ascorbic acid from degrading (Güneș & Dölek, 2010). In our results, higher altitudes exhibits significant higher vitamin C concentration due to the decreased in oxygen concentration which reduces degradation of vitamin C in rosehip (Roman et al., 2013).

 The current study showed that as the altitude increases, the concentration of phenol and flavonoids also increases. Numerous researchers have reported that UV-B radiation increases at higher altitudes, which is directly proportional to increase in plants phenolic and flavonoid contents (Chen et al., 2019). Total phenols and flavonoids are responsible for plant growth and development. Furthermore, these antioxidants are responsible for the plants to survive under high UV-B radiation (Ekiert et al., 2022). The increased production of flavonoids and phenols in plants at high altitudes can be attributed to UV-B radiation (Živković et al., 2021). Phenolic compounds with a hydroxyl group exhibit antioxidant properties. Phenolic compounds are an essential class of molecules found in plant material. Due to the presence of the hydroxyl group, these compounds play a crucial role in providing antioxidant properties to plants. The primary source of phenolic compounds' antioxidant potential is their redox characteristics, which enable them to function as metal chelators, hydrogen donors, reducing agents, and singlet oxygen quenchers. It seems that phenolics are the primary phytochemicals that give plant materials their antioxidant properties.

 Phenolics are considered as the primary phytochemicals which are responsible for the antioxidant activity of plant materials (Balasundram et al., 2006). By using Pearson's correlations, we confirmed the relationship between the total phenol concentration and the total antioxidant capacity (Table 3). With an \mathbb{R}^2 value of 0.2126 and a significance level of *P*≤0.05, these results showed that there is a strong positive connection between the total phenol content and the total antioxidant activity of extracts derived from wild roses. These findings suggest a potential link between the concentration of phenolic hydroxyl

groups in each extract and its overall antioxidant activity. The antiradical activity of phenolic compounds is influenced by several factors. These include the molecular structure of phenolic compounds, the availability of phenolic hydrogen, the ability to stabilize phenoxyl radicals created by hydrogen donation, and the polarity of the extraction solvent, which affects the extractability of the compounds (Roman et al., 2013). Several other studies have found positive correlation between total phenols and antioxidant activity in various wild fruits (Kılıçgün & Altıner, 2010; Egea et al., 2010). The results of our study indicate that a high phenolic content is associated with high antioxidant activity (Table 3). These results are consistent with reports on a number of medicinal plant species, including *Momordica charantia*, *Strobilanthes crispa*, *Polygonum minus*, *Andrographis paniculata*, *Hedychium spicatum*, *Valeriana jatamansi*, and *Torilis leptophylla*. These reports showed a positive correlation between total phenolic content, flavonoid content, and antioxidant activity (Qader et al., 2011; Rawat et al., 2011; Saeed et al., 2012; Jugran et al., 2016). Phenolic compounds act as antioxidants due to their ability to donate electrons and stabilize radical intermediates (Kumar et al., 2006). Plant phytochemical composition and antioxidant activity vary with altitude due to changing environmental conditions (Adhikari et al., 2022).

Fig. 2 Total flavonoids (mg/100 g FW) (Fig. 2a), total phenols (mg/100 g FW) (Fig. 2b), total anthocyanins (mg of cyanidine-3-glucoside/100 g FW) (Fig. 2c) and total antioxidants activity (μ g/100 g FW) (Fig. 2d) of wild rose collected from four different locations of Rawalakot.

Conclusion

Based on the current study, it can be concluded that wild roses collected from higher elevations, specifically Khai Gala and Chota Gala, demonstrated superior growth, floral, and biochemical characteristics compared to those from lower elevations. This suggests that altitude plays a significant role in the development and quality of wild roses. These findings highlight the potential advantages of cultivating wild roses at higher elevations, which may lead to enhanced ornamental and commercial value. However, it is important to note that while the study provides valuable insights, further research is necessary to explore additional parameters that could influence the growth and quality of wild roses. Future studies should aim to identify more specific factors contributing to the observed differences and assess the feasibility of large-scale cultivation. Such research would be essential to fully exploit the commercial potential of wild roses, ensuring sustainable and profitable production practices. The study establishes a clear link between elevation and the enhanced characteristics of wild roses, paving the way for future investigations to optimize cultivation techniques and maximize the economic benefits of these valuable plants.

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