Morphological Characterization and Phytochemical Analysis of Moringa Plant (*Moringa oleifera* L.) Different Altitudes in Bali

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Abstract. The Moringa plant (Moringa oleifera L.) is a plant that has many benefits and has long been used by various groups throughout the world. Almost all parts of this magical plant can be used for multiple purposes. Moringa plants spread and adapt to different environments, increasing phenotypic and phytochemical diversity. The research was conducted at three altitudes in Bali, lowlands, medium, and highlands from December 2023—May 2024. The study was conducted to determine differences in morphological characters and phytochemical content of Moringa plants. This research used UPGMA analysis to determine the relationship based on morphological characters, flavonoids, tannins, and vitamin E using a UV-Vis Spectrophotometer, while the antioxidant activity test was carried out using the DPPH method. The results of the identification of morphological characters showed differences in qualitative characters, which were seen in leaf color and flower color, while quantitative characters were seen in leaf length, leaf thickness, pod length, and number of seeds. Nine Moringa accessions showed differences in phytochemical content. The highest flavonoid and vitamin E content in the lowlands are (599.961 mg QE/100 g) and (599.961 mg QE/100 g), tannins in the medium plains (368.140 mg TAE/100 g), and the highest IC50 in the highlands (54.94 ppm).

Keywords: altitude impact; antioxidant activity; Moringa oleifera; morphological diversity; phytochemical

INTRODUCTION

The Moringa oleifera L. plant is known for its numerous health and nutritional benefits. It is often referred to as the "miracle tree" because almost all parts of the plant, from the leaves and seeds to the flowers and roots, possess high nutritional and medicinal value. Moringa contains high levels of protein, vitamins, and minerals, as well as phytochemical compounds flavonoids, saponins, and tannins, which potential as antioxidants, have antiinflammatory agents, and antimicrobial agents (Muhl, 2016; Tshabalala, 2020).

The morphological and phytochemical diversity of Moringa plants is strongly influenced by various environmental factors, one of which is altitude. Altitude can influence climatic conditions such as temperature, humidity, and light intensity, which in turn can affect plant growth and phytochemical content. Therefore, research on morphological characterization and phytochemical analysis at various altitudes is

crucial to understanding how these environmental factors influence the quality and quantity of phytochemical components in Moringa plants (Rachmawatie et al., 2022; William et al., 2014).

Bali, as one of the regions in Indonesia with significant altitude variation, is an ideal location for this research. With its varying altitudes from coastal areas to mountainous areas. Bali offers diverse environmental conditions to study the effect of altitude on the morphology and phytochemical content of moringa plants. Morphological and phytochemical diversity in a plant occurs when the same plant's genetic makeup is grown in different locations. Morphological diversity directly reflects the plant's characteristics (Sobari & Wicaksana, 2017; Usman et al., 2021).

To date, no research has examined the morphological diversity and phytochemical content of moringa plants at different altitudes in Bali. Therefore, this study aimed to explore the differences in morphological



characteristics and phytochemical content of moringa plants grown at different altitudes in Bali. This research will not only provide useful scientific information but also assist farmers and health practitioners in optimizing moringa cultivation and utilization in Bali.

METHODS

identification of **Exploration** and Moringa plants were carried out in lowland, medium, and highland areas in Bali, while phytochemical analysis was conducted at the **Mathematics** and Natural Sciences Laboratory of Udayana University, which took place in December 2023-March 2024. The tools and materials used were analytical scales, mucus, measuring flasks, calipers, UV-vis spectrometers, cameras, instruments, Moringa leaf powder, magnesium powder, HCl, FeCl3, 90% ethanol, DPPH Whatman filter paper, aluminum foil, plastic wrap, and tissue rolls.

UPGMA analysis was used to examine kinship relationships based on morphological characters, flavonoids, tannins, and vitamins using a UV-Vis Spectrophotometer. Antioxidant activity was measured by the DPPH method. Quantitative morphological data were analyzed using ANOVA.

RESULTS AND DISCUSSION

This study obtained moringa plants from lowland, midland, and highland areas, providing a deeper understanding of the morphological differences and phytochemical content of moringa (Moringa oleifera L.) grown at different altitudes in Bali. The results of the Moringa kinship analysis using the UPGMA method with a Similarity Index showed clusters clustered on a scale of 44.52 to 1. A Similarity Index of 44.52 or 44.52% formed two clusters, A and B, with a similarity below 50% (Figure 1).

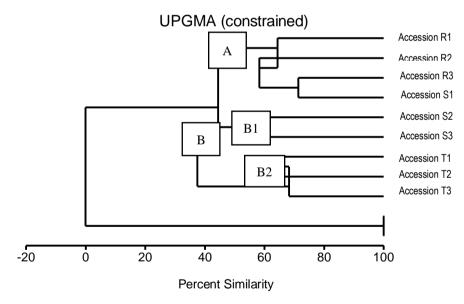


Figure 1. Dendrogram of qualitative morphological characters

According to <u>Darmawati (2020)</u>, two or more objects are categorized as morphologically similar if their similarity is greater than 50%. Therefore, the qualitative or phenotypic relationship between these nine Moringa accessions is categorized as distantly similar, as the similarity between clusters A and B is below 50%. This is

supported by differences in leaf color. The leaves in the lowlands and midlands are darker green compared to the lighter green in the highlands (Figure 2). According to Ajhar et al. (2018), Moringa leaf color is more influenced by genetic traits than by the growing environment. This aligns with Wulantika (2019), who stated that qualitative

traits such as Moringa leaf and flower color are controlled by a single major gene, a gene with a significant influence, so environmental influences are minimal.

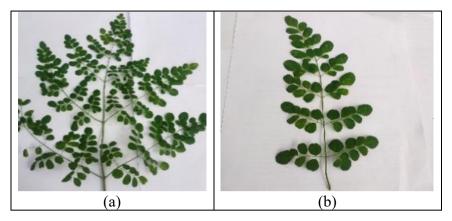


Figure 2. Moringa leaves from the lowlands (a) and highlands (b)

The results of the kinship relationship of Moringa plants from low, medium and high plains identified based on quantitative morphological characters in several variables, namely tree height, stem diameter, leaf length, leaf width, leaf thickness, leaf blade length, leaf blade width, number of leaf veins, flower stalk length, number of stamens, stamen length, number of petals, pod length, and number of seeds, show that all accession samples are separated by two large clusters A and B with a similarity level below 50%, namely 40% (Figure 3).

According to Abdulazeez et al., (2025), the growing environment greatly influences the determination based on morphological markers. Environmental conditions and lack of nutrients for Moringa plants greatly influence the differences in tree height, stem diameter, One of the environmental factors that influence plant growth is altitude, in line with the results of research that has been done, the highest data obtained from each variable such as plant height, leaf length, leaf width, leaf thickness, leaf blade length, leaf blade width, number of flowers, flower length, flower width, pod length and the highest number of Moringa seeds were obtained from the lowlands of each variable in Moringa accessions. According to Ayu et al. (2016) stated that optimal vegetative growth in a plant variety is due to the variety having adapted to its growing environment and in line with Lubis (2017) that plant response to the environment can be seen from the increase or decrease in plant size which is a combination of genetic and environmental factors determined by climate, weather, temperature and nutrient composition in the soil. One environmental factor influencing plant growth is altitude. Research has shown that the highest data for each variable, such as plant height, leaf length, leaf width, leaf thickness, leaf blade length, leaf blade width, flower number, flower length, flower width, pod length, and moringa seed number, are found in lowlands. Environmental factors that support optimal plant growth include water availability, better warmer temperatures, richer soil nutrients, lower wind pressure, and a longer growing season.

According to Malhi et al. (2020), rainfall is often higher in lowlands and water distribution is more even than in highlands. These conditions provide trees with better access to water, which is crucial for tree height growth and the development of other organs. Furthermore, warmer temperatures in lowlands support higher photosynthetic activity and faster metabolism. Warmer temperatures also reduce the risk of damage from frost or extremely low temperatures, which often occur in highlands. Anderson (2020) added that soil nutrients are also a influencing plant growth development.

Meanwhile, in mid- and highland areas, temperatures are lower and temperature fluctuations between day and night are greater. Low temperatures slow the rate of photosynthesis and plant metabolism, which directly impacts tree growth. Furthermore, the risk of frost can cause damage to plant tissue (Johnstone, 2021). Bruelheide et al. (2018) and Scholten et al. (2017) stated that soil in highlands is often thinner, rockier, and

less fertile, resulting in lower nutrient availability. Furthermore, water drains more quickly on sloping land, so trees in highlands often experience water shortages that inhibit growth. Furthermore, highlands also receive more UV radiation due to the thinner atmosphere. The varying environmental conditions where plants grow can affect the content of secondary metabolites, such as phytochemicals.

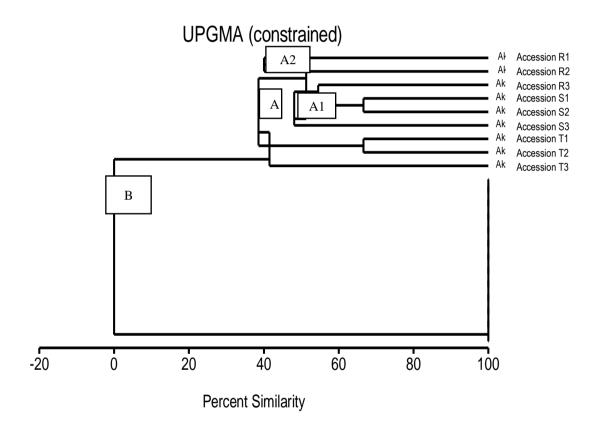


Figure 3. Dendrogram of quantitative morphological characters

The highest flavonoid content of moringa leaves was obtained in the lowland R3 accession of 599.97% compared to the medium land S1 accession of 209.75% in accordance with the statement of Safa et al. (2014), that the total flavonoid content is considered high if the value obtained is >70 mg GAE/g. The flavonoid phytochemical content produced with high concentrations can be influenced by environmental factors. One of the most important environmental factors is the altitude where the moringa plant grows. From this study, it can be seen that

altitude can affect the Flavonoid content of moringa leaves; the highest flavonoid content is obtained from the lowlands. Lowlands have optimum environmental factors for the formation of secondary metabolites. especially flavonoids in moringa plants, lowlands have the highest temperature and light intensity compared to medium and highlands, so it is possible that the process of photosynthesis and flavonoid biosynthesis at this altitude can run optimally. Lowlands have the highest temperature and light intensity compared to medium and highlands,

so it is possible that the process of photosynthesis and flavonoid biosynthesis at this altitude can run optimally. Flavonoid biosynthesis is produced through a combination of shikimic acid and malonic acid pathways, both of which are derived from carbohydrates through the glycolysis process (Julianti et al., 2021). The highest tannin content was obtained by accession S1 and there was an increase of 368.14% compared to accession R1 (Table 1).

Tannins are often produced in response environmental stress. including to temperature, light intensity, water availability, plant-microorganism and interactions. According to Dixon & Paiva (1995), in temperate latitudes, temperatures are usually less extreme than in cooler highlands or hotter lowlands. These moderate temperatures can increase enzymatic activity that plays a role in tannin synthesis. <u>Kitamura</u> (2022) added that in temperate latitudes, light intensity is usually quite high but less extreme.

Sufficient light increase can photosynthesis, which then provides more precursors for the synthesis of secondary compounds such as tannins. important factor is water. Guo et al. (2021) stated that insufficient water availability in temperate latitudes can cause mild stress in plants. This stress can stimulate tannin production as part of the plant's defense mechanism against dehydration or pathogen attack. Meanwhile, the highest vitamin E content was obtained in the highland T2 accession at 82.85% compared to the lowland R2 accession at 17.39% (Table 1). Vitamin E, or tocopherol, is an important antioxidant that protects plant cells from oxidative damage.

Table 1. Flavonoid, Tannin, and Vitamin E levels of Moringa accessions at three different altitudes in Bali

No	Moringa accession	Flavonoid (mg QE/100 g)	Tannin (mg TAE/100 g)	Vitamin E (mg tocopherol/100 g)
1	R1 accession	398.406	158.675	66.810
2	R2 accession	356.266	308.499	17.393
3	R3 accession	599.961	350.227	79.115
4	S1 accession	209.754	368.140	29.354
5	S2 accession	548.867	326.835	58.722
6	S3 accession	386.373	299.659	79.267
7	T1 accession	277.359	323.300	58.056
8	T2 accession	239.781	280.458	82.858
9	T3 accession	296.093	279.800	65.285

Description: R1-R3: moringa samples from lowlands, S1-S2: moringa samples from midlands, T1-T3: moringa samples from highlands, QE: Quercetin Equivalent, TAE: Tannic Acid Equivalent

Vitamin E content is often higher in plants grown at high altitudes compared to low or medium altitudes due to the influence of UV radiation intensity at higher altitudes, due to the thinner atmospheric layer. Higher UV exposure encourages plants to produce more antioxidants, including vitamin E, as a

protective mechanism against UV damage Li & Schemske (2021), then Espley & Jaakola (2023) added that at high altitudes, plants often experience more extreme stress conditions, such as colder temperatures and greater temperature fluctuations between day and night. This higher oxidative stress

stimulates increased vitamin E production to protect plant cells from oxidative damage. Lower temperatures at high altitudes can induce a stress response in plants. One adaptive response of plants is to increase the synthesis of antioxidant compounds such as vitamin E. Lower air pressure at high altitudes reduces oxygen availability, which can affect plant metabolism and stimulate the production of protective compounds such as vitamins. Plants grown at high altitudes have also often experienced natural selection that encourages plants to develop higher levels of antioxidants as part of adaptation to harsher environments (Meng et al., 2025). In contrast to the flavonoid, tannin, and vitamin E

content, the highest antioxidant activity (IC50) of moringa leaves was found in the highland moringa accession code T9, at 54.94 ppm, but it has a strong antioxidant activity category compared to other accessions that have low IC50 values but have very strong activity categories. antioxidant antioxidant activity in this study showed that the lowland moringa accession code R3 contained the highest flavonoid content with a value of 599.961 mg QE/100 g, but had a low IC50 result of 43.79 ppm, compared to the T9 moringa accession which had the lowest flavonoid value at 296.093 mg QE/100 g, but showed the highest IC50 concentration value at 54.94 ppm (Table 2).

Table 2. Antioxidants (IC50) of kolor accessions at three different altitudes in Bali

No	Accession	IC ₅₀ value	Antioxidant activity
		(ppm)	categories
1	R1	41.11	Very strong
2	R2	24.49	Very strong
3	R3	43.79	Very strong
4	S1	31.92	Very strong
5	S2	39.33	Very strong
6	S3	44.82	Very strong
7	T1	32.70	Very strong
8	T2	54.94	Strong
9	T3	39.20	Very strong

According to Siswoyo et al. (2016), one of the influencing factors is the environment in this case the height of the growing place of the Moringa plant that the high calcium (Ca) nutrient around the plant can function as an enzyme activator by affecting the secondary metabolite pathway, so that the flavonoid content in the leaves of tabat barito (Ficus deltoidei) increases and is able to influence the process of antioxidant activity supported by Sulasiyah et al. (2018), stating that one of the important influences in increasing antioxidant activity is the flavonoid content, this content is the strongest main compound that plays an active role as an antioxidant in the phenol group and in line with Nur et al. (2019) that there is a correlation between flavonoids and antioxidant activity, such as

the flavonoid content which has a significant positive correlation with antioxidant activity.

CONCLUSION

Qualitative morphological characters show differences in lowland, medium, and highland moringa, showing differences in leaf and flower color. Quantitative characters show differences in plant height, leaf length, leaf width, leaf thickness, pod length, and number of seeds. The kinship relationship morphological based on qualitative characters shows that lowland moringa, namely R1, R2, R3, and midland moringa, namely S1, form a separate cluster with a similarity index of 58.33%. Meanwhile, quantitative morphological characters show that highland moringa, T1, T2, and T3, form

a separate cluster with a similarity level of 67%. In general, the data from the UPGMA analysis, both quantitative and qualitative, show that altitude influences morphological differences. 2. The highest flavonoid and vitamin E content in the lowlands is (599.961 OE/100 and (88.858) mg g) TOCOPHEROL/100 g), the highest tannin in the medium plains is (368.140 mg TAE/100 g), and the highest antioxidant activity (IC50) in the highlands is 54.94 ppm. relationship between morphological and phytochemical characteristics in Moringa leaves varies based on altitude. In the lowlands, the leaves tend to be thicker and greener due to the high light intensity and hot temperatures that stimulate leaf growth and flavonoid production.

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