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Effect of Different Altitudes on Morpho-Physiological Attributes Associated with Mango Quality

Cuixian Zhang †, Huaifeng Yi †, Xianyu Gao, Tianqi Bai, Zhangguang Ni, Yufu Chen, Meicun Wang, Yong Zhang, Jihong Pan, Weilin Yu and Dehong Xie *

Institute of Tropical and Subtropical Cash Crops, Yunnan Academy of Agricultural Sciences, Baoshan 675800, China
* Correspondence: rjsxdh@yaas.org.cn
† These authors contributed equally to this work.

Abstract: Mango (*Mangifera indica* L.) is a widely cultivated fruit in tropical and subtropical areas at altitudes ranging from 100 to 1500 m above sea level (masl). However, little is known about the effects of altering altitudes on the morpho-physiological traits determining the commercial value of mango. Therefore, we systematically investigated a commercial mango cultivar at eight altitudes ranging from 680 to 1400 masl to check the environmental impact on morpho-physiological attributes and volatile compounds using analysis of variance, principal component analysis, clustering, and correlation. We observed an increase in fruit weight and size from 680 to 1000 masl elevation and a gradual decrease at higher altitudes above 1000 msl. In contrast, quality parameters, including total soluble solids and total sugar, decreased with the increase in altitude, while the total acid increased with the increase in altitude. Moreover, we characterized the dried fruit, pericarp, and sarcocarp for aromatic compounds and identified 110 volatile compounds. The accumulation pattern of the volatiles suggested a considerable influence of environmental factors associated with altering altitudes. However, there was no clear trend in the volatile accumulation at different altitudes. We further determined the ten most frequently occurring volatiles at different altitudes and tissues. For instance, Alpha-Guaiene was only identified at 1000–1215 masl altitudes in dried fruit, while Beta-Ocimene showed the highest accumulation at 900 masl in dried fruit and pericarp. Together, our study provides clues on the impact of the altitude on mango fruit yield and quality attributes, which will guide future agronomic practices.

Keywords: mango; altitude; volatiles; quality; yield

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1. Introduction

Mangifera indica, the king of fruits, is widely cultivated worldwide in tropical and subtropical areas [1]. India, China, Thailand, Indonesia, and Pakistan are the top five producers of mango in the world, with India producing approximately 50% of the mangoes in the world [2]. Its wider adaptability helped this tree to establish itself in various agro-climatic zones [3,4]. The main mango-producing areas in China are Hainan, Guangxi, Yunnan, and Sichuan, with Taiong No.1, Guifei, Jinhuang, Mya HinTa, Keitt, Sensation, Chok Anan, Nam Doc Mai 4, and Xiangya as the main varieties in different regions [5,6].

Geospatial parameters such as temperature, humidity, and altitude significantly control growth rate and fruit development. In the tropics, the mango grows almost anywhere up to 1200 m elevation, however, for fruit production, a prominent dry season lasting more than three months is necessary [7,8]. Overwhelming reports suggest a decrease in growth with poor productivity at more than 1000 m elevation above sea level, and the 600 m threshold is considered optimal for commercial production [4,9–13]. A flowering flush is produced during the dry season, but on the contrary to the subtropics, flowering is erratic and a yield-limiting factor [14].

Quality and aroma, characteristic traits of the fruit, depend on the peel, seed traits, soluble solids, total acid, total sugars, and primary and secondary metabolites [15–18]. All these traits are largely modified under changing growing conditions. As the altitude increases, the temperature, relative humidity, rainfall, soil structure, and the texture change [19]. The volatile compounds affecting mango fruit quality and aroma are well known and vary across genotypes and cultivation areas [20–22]. For instance, Pino et al. [22] emphasized ethyl-2-methylpropanoate, ethyl butanoate, (E,Z)-2,6-nonadienal, €-2-nonenal, methyl benzoate, €-β-ionone, decanal, and 2,5-dimethyl-4-methoxy-3 (2H)-furanone as main volatiles contributing towards overall aroma. However, little is known about the influence of environmental factors at higher altitudes on fruit quality. In mangoes, metabolites are produced during the development of the fruit and play a crucial role in fruit maturity, aroma, and abiotic stress resistance. Specific metabolic pathways, such as the phenylpropanoid pathway and sugar signaling, derive from precursors of primary metabolism and play a key role in the plant's interaction with the environment [23]. Secondary metabolites present in the fruit protect it against biotic and abiotic stresses. For instance, phenol, terpenoid, and flavanol help keep the fruit safe from bacteria with their antibacterial and antioxidant properties [24,25]. Studying secondary metabolites in mango is of great importance as they directly affect the yield, quality, and aroma.

The diversity of mango fruits is enormous, and each variety has a unique flavor and characteristics [15,26–28]. A mango variety is characterized by plant growth, young and mature leaf color, stone size, inflorescence color, and fruit traits such as color, skin, size, shape, taste quality, aroma, pulp content, and fruit mass [29–32]. 'Myahintha' mango, an established variety in Yunnan province (China), was used as a test subject in this study. 'Myahintha' belongs to the Anacardia family, the genus Mango, and is one of the main mango varieties in Yunnan. It was introduced from Myanmar in 2002 by the Tropical and Subtropical Economic Crops Research Institute of the Yunnan Academy of Agricultural Sciences. The current planting area in Yunnan exceeds 30,000 acres. The climate types of Yunnan's hot areas are diverse. The hot zones are distributed from the lowest 76 to the highest 1600 m above sea level (msl). The territory is mountainous, with elevations ranging from 300 to 1600 msl. It has the characteristics of sufficient sunlight, high temperature throughout the year, and distinct dry and wet seasons, a unique high-quality mango-producing area in the country. In recent years, due to the high economic benefits of growing Myahintha mangoes, different ecological regions of Yunnan were introduced and tested. The production and planting areas have gradually expanded from low-altitude to high-altitude areas.

An increase in altitude exposes mango plants to low temperatures and more hard soils, resulting in chilling injury and reduced vegetative growth. The study of morpho-physiological and quality attributes in mango at different altitudes is, therefore, of great importance. In this study, we aimed to characterize the main mango variety in Yunnan, Myahintha mango, for morpho-physiological traits and differential accumulation of aromatic compounds at different altitudes.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

'Myahintha' was used as a test subject in this study to characterize morphological and quality traits. The dataset and samples for further downstream analysis were collected from established mango grooves in Lujiang, Baoshan, Yunnan Province, at eight different altitudes, including 680, 800, 900, 1000, 1100, 1215, 1300, and 1400 msl. They are located in the dry–hot valley of the Nujiang River. The average annual temperature is 21.3 °C. The average temperatures of the hottest and coldest months are 26.4 °C and 13.9 °C, respectively. The highest and mildest temperatures are 40.3 °C and 0.2 °C, respectively; the sunshine hours are 2329.7 h, and the annual average radiation is 138,449 cal/(year·cm^2); the annual average rainfall is 755.3 mm with the rainy season (May–October) and dry season (November–April); the precipitation is 614.7 mm and 132.8 mm, respectively, accounting for 82% and 18% of the annual precipitation and the annual evaporation is 2039.8 mm. The soil quality of the test site is with medium fertility.

The samples for downstream analysis were collected when the mango trees entered the abundant production period with vigorous growth; all were eight years old. The collection time was from July to September, when fruit ripeness reached 80%.

2.2. Sample Preparation and Data Collection

We randomly selected three naturally ripened fruits. Pericarp (peel and pulp) and sarcocarp (pulp) tissues from each sample were homogenized separately with a juice machine and stored at −20 °C for later use. Similarly, for dried fruit, we randomly selected three naturally ripened fruits. The fruits were peeled off and cut into slices with a thickness of 1 cm. The slices were dried in an oven at 65 °C for 24 h and crushed into powder for further use.

Moreover, the morphological characterization of five randomly selected fruits was performed using the following traits, including fruit weight (Fwt), fruit long diameter (FLD), fruit trans diameter (FD), fruit thickness (FT), peel weight (Pwt), seed weight (Swt), seed length (SL), seed width (SW), and seed thickness (ST).

Total soluble solids (TSS), total acid (Acon LS), and total sugar (SuCon) were measured following the procedure reported by Makroo et al. [33].

2.3. Identification and Characterization of Volatile Compounds

The volatile compounds from dried fruit, pericarp, and sarcocarp were identified using Gas Chromatography-Mass Spectrometer 7890A-5975C, Agilent Company, Beijing, China. For each sample, a 5.0 g was placed in a headspace bottle, sealed, and kept in a 40 °C water bath. Extraction was performed for 30 min, and then the extraction head was inserted into the inlet of the gas-mass spectrometer. GC conditions were kept as: starting temperature 60 °C, heating up to 120 °C at a rate of 4 °C/min, running for 16 min, then heating up to 200 °C at a rate of 5 °C/min, keeping for 3 min; inlet temperature 250 °C. The carrier gas was 99.99% helium gas, and the flow rate was kept at 1.2 mL/min. MS conditions were as follows: electron bombardment energy was 70 eV, ion source temperature at 230 °C, transmission interface temperature at 250 °C, scanning range was 35~350 *m/z*, and MS quadrupole temperature was 150 °C.

For downstream analysis, we used the NIST17. L mass spectral standard library to automatically search and control the mass spectrum data and determine the chemical composition according to the molecular formula and chemical synthesis database number of each substance.

2.4. Data Analysis

Data for phenotypic and quality characteristics were analyzed using the least square test, which takes advantage of the best fit for a set of data points by minimizing the sum of the offsets or residuals of points from the plotted curve [34]. The least-square analysis of variance was performed to understand the variation within the datasets.

Principal component analysis, cluster analysis, and Pearson's correlation were performed using default and standardization options with SAS-JMP Pro 16 (SAS Institute Inc., Cary, NC, USA, 1989–2021).

3. Results

3.1. Phenotypic Characterization

To characterize the phenotypic variation and quality attributes of mango at different altitudes, collected data were subjected to analysis of variance. Parameters including Fwt, FLD, FD, FT, Pwt, Swt, SL, SW, ST, TSS, Acon LS, and SuCon were analyzed across eight altitudes. Highly significant differences were observed among different altitudes for all the aforementioned traits (Table 1). The phenotypic variation attributed to the altitude was further evaluated using principal component analysis (PCA), correlation analysis, and cluster analysis. Principal component analysis was performed on the multivariate data to obtain a smaller set of variables (summary indices) explaining variation, trends, clusters, and outliers. PCA distributed variation in twelve principal components, but only two components were important as they had an eigenvalue higher than one. Component 1 had an eigenvalue of 7.269 and accounted for 60.6% of the variance. The second component showed an eigenvalue of 2.2188, accounting for 18.5% of the variance. Overall, these three components explained the cumulative variability of 79.1% (Figure 1C). The scatter plot of the principal component depicted that altitudes of 680 m, 1100 m, and 1215 m were in close proximity and positive ordination (Figure 1B,C). In contrast, altitudes of 800 m, 900 m, and 1000 m were in the first upper ordinate, whereas 1200 m and 1300 m were in the first lower portion of the quadrate. The nonlinear trends, for instance, the proximity of altitudes of 680 m with 1100 and 1215 m, are intriguing. However, this needs further validation using a detailed analysis of environmental attributes such as temperature, rainfall, and soil types. The loading matrix, depicting the contribution of each trait to the corresponding PC, suggested that traits FLD, FD, and SL have the most significant impact on PC1, followed by Fwt, Pwt, SW, FT, Swt, and Acon (Table S1). While TSS and SuCon were a major influence on PC2, ACon, and SW were in close proximity but in opposite directions to TSS and SuCon. These traits seem to be contributing to the variance in the dataset. The fruit, peel, and seed traits are all gathered in one ordinate and very close to each other—except for SW.

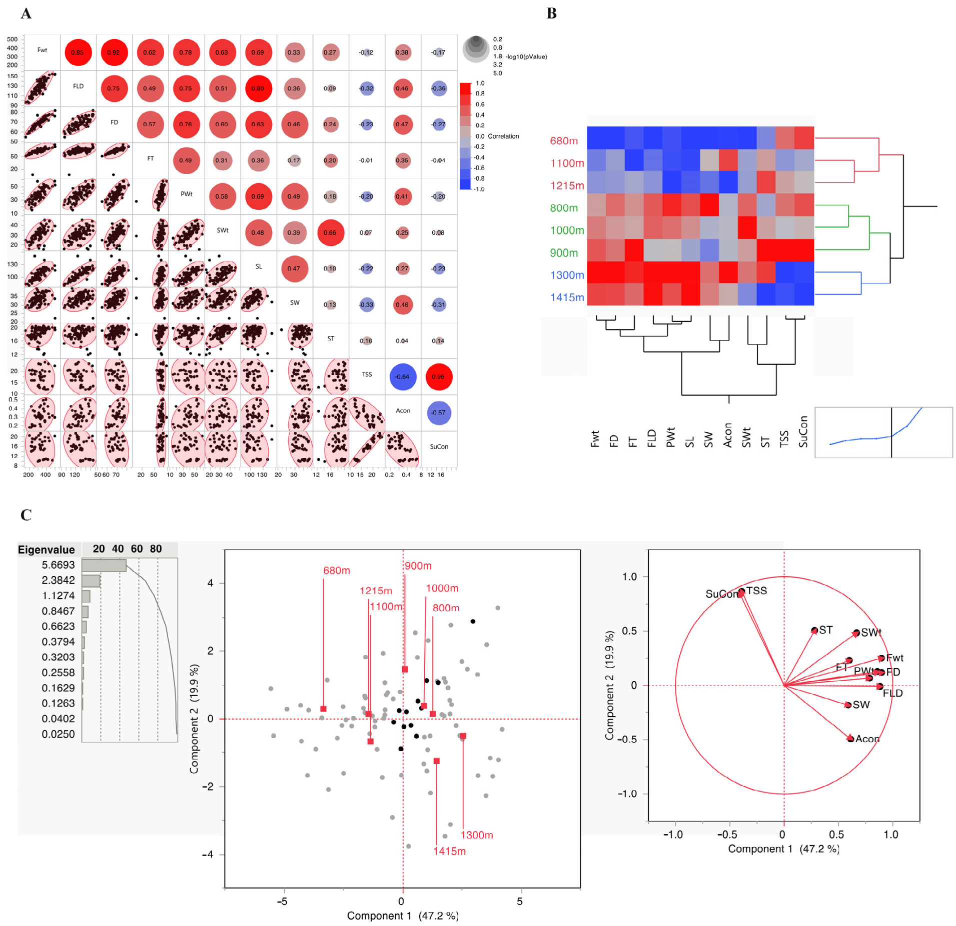

Figure 1. Multivariate analysis of phenotypic and quality traits: (**A**) Pearson's correlation between all traits. Diagonal is labeled as traits, and on upper triangle shows the pairwise correlation between traits, and the lower triangle shows the distribution of each trait (values of the x axis and y axis are traits values according to which the distribution pattern was estimated); (**B**) Clustering based on phenotypic traits and altitude. The graph in the bottom right corner shows the number of significant clusters based on *F*-statistics; and (**C**) Principal component analysis depicting the distribution of traits and locations based on the first two components, PC1 and PC2.

Table 1. Analysis of variance (least square fit model) table showing mean square values and the significance of different parameters pooled over different altitudes.

Sources	Fwt	FLD	FD	FT	Pwt	Swt	SL	SW	ST	TSS	Acon	SuCon
Altitudes	29,033	1380.2	169.24	173.79	492.05	200.71	677.06	41.647	6.66	67.165	0.039	71.810
Error	2567	104.15	13.704	48.521	47.346	27.761	112.17	6.7952	2.72	0.1854	0.001	0.3819
Signifi.	**	**	**	**	**	**	**	**	*	**	**	**

fruit weight (Fwt); fruit longitudinal diameter (FLD); fruit transversal diameter (FD); fruit thickness (FT); peel weight (Pwt); seed weight (Swt); seed length (SL); seed width (SW); seed thickness (ST); total soluble solids (TSS); total acid (Acon LS); total sugar (SuCon). Where * indicates significance at $p < 0.05$ and ** indicates $p < 0.01$.

Moreover, we estimated Pearson's correlation among all morphological traits and identified that the fruit weight (Fwt) showed a high correlation value with FLD, FD, FT, Pwt, Swt, and SL, whereas it showed a weak positive correlation value for SW, ST, and ACon (Figure 1A). Fwt showed a weak negative relation with TSS and SuCon. Peel weight (Pwt) showed a high positive correlation with Fwt, FLD, FD, and FT. This showed a medium correlation value with Pwt, SL, Swt, and ACon, whereas it depicted a weak negative correlation with TSS and SuCon. Whereas SW showed a medium to weak correlation coefficient with all fruit, seed, and peel traits, it showed a weak negative correlation with TSS and SuCon. TSS had only a high positive correlation with SuCon. It showed a weak positive correlation with Swt and a negative correlation with ACon, SW, SL, Pwt, FT, FD, FLD, and Fwt.

The mean performance of all the traits was represented in Figures 2 and 3. Most morphological attributes were lower at 600 m elevation with a significant increase up to 1000 m elevation and gradual decrease above 1000 m elevation (Figure 2). This differential pattern can be attributed to the change in environmental parameters at higher altitudes. However, quality parameters, including total soluble solids (TSS) and total sugar (SuCon), depicted a continuous decrease with the increase in alleviation, while total acid (Acon LS) increased at higher altitudes (Figure 3).

3.2. Characterization of Volatile Compounds

Changes in volatiles composition could induce alterations in flavor and aroma. To comprehend the effect of increasing altitudes on aromatic compounds, we characterized dried fruit, pericarp, and sarcocarp tissues for available volatiles. The accumulation pattern of volatile compounds in the three tissues was highly differentiated, with no obvious accumulation pattern between different altitudes. As a result, we identified a total of 104 volatile compounds at least accumulated in one tissue (dried fruit, pericarp, and/or sarcocarp) (Table S2).

Volatile compounds, including alpha-Guaiene, beta-Ocimene, 136-Octatriene, 37-dimethyl-(Z)-246-Octatriene, 26-dimethyl-(EZ), 26-Dimethyl-1357-octatetraene-EE, Az-ulene-1233a4567-octahydro-14-dimethyl-7-(1-methylethenyl), Azulene-12356788a-oc- tahydro-14-dimethyl-7-(1-methylethenyl), Cyclohexasiloxane, dodecamethyl, Cyclopen- tasiloxane, decamethyl, and trans-beta-Ocimene were identified as most frequent occur- ring in dried fruit, pericarp, and sarcocarp at 680 m, 800 m, 900 m, 1000 m, 1100 m, 1215 m, 1300 m, and 1400 m (Figure 4 and Table S3). Alpha-Guaiene was identified in dried fruit at 1000–1215 m altitude, while in pericarp, it was only identified at 680 m, 1000 m, 1215 m, and above. Moreover, alpha-Guaiene was not identified in sarcocarp. Beta-Ocimene depicted a differential accumulation pattern in dried fruit, pericarp, and sarcocarp at all the studied altitudes. The highest accumulation of beta-Ocimene was identified at 900 m elevation in dried fruit and pericarp. 136-Octatriene-37-dimethyl-(Z) also depicted a differential accumulation pattern with the highest accumulation (9.47×10^7) at a 900 m elevation in dried fruit and pericarp tissues, while it was at its lowest in sarcocarp tissues. The other volatiles, including 246-Octatriene, 26-dimethyl-(EZ), 26-Dimethyl-1357-oc- tatetraene-EE, Azulene-1233a4567-octahydro-14-dimethyl-7- (1-methylethenyl), Azulene-12356788a-octahydro-14-dimethyl-7-(1-methylethenyl), Cyclohexasiloxane dodecame- thyl, Cy-clopentasiloxane decamethyl, and trans-beta-Ocimene were also identified with a differential regulation pattern. Azulene-1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) and Azulene-1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) were not identified in sarcocarp tissues at any altitude. The remaining less frequent volatiles were identified with accumulation patterns in at least one tissue at one altitude under study. However, no apparent trend was identified in the accumulation pattern of volatiles at different altitudes, requiring further study with multiple genotypes.

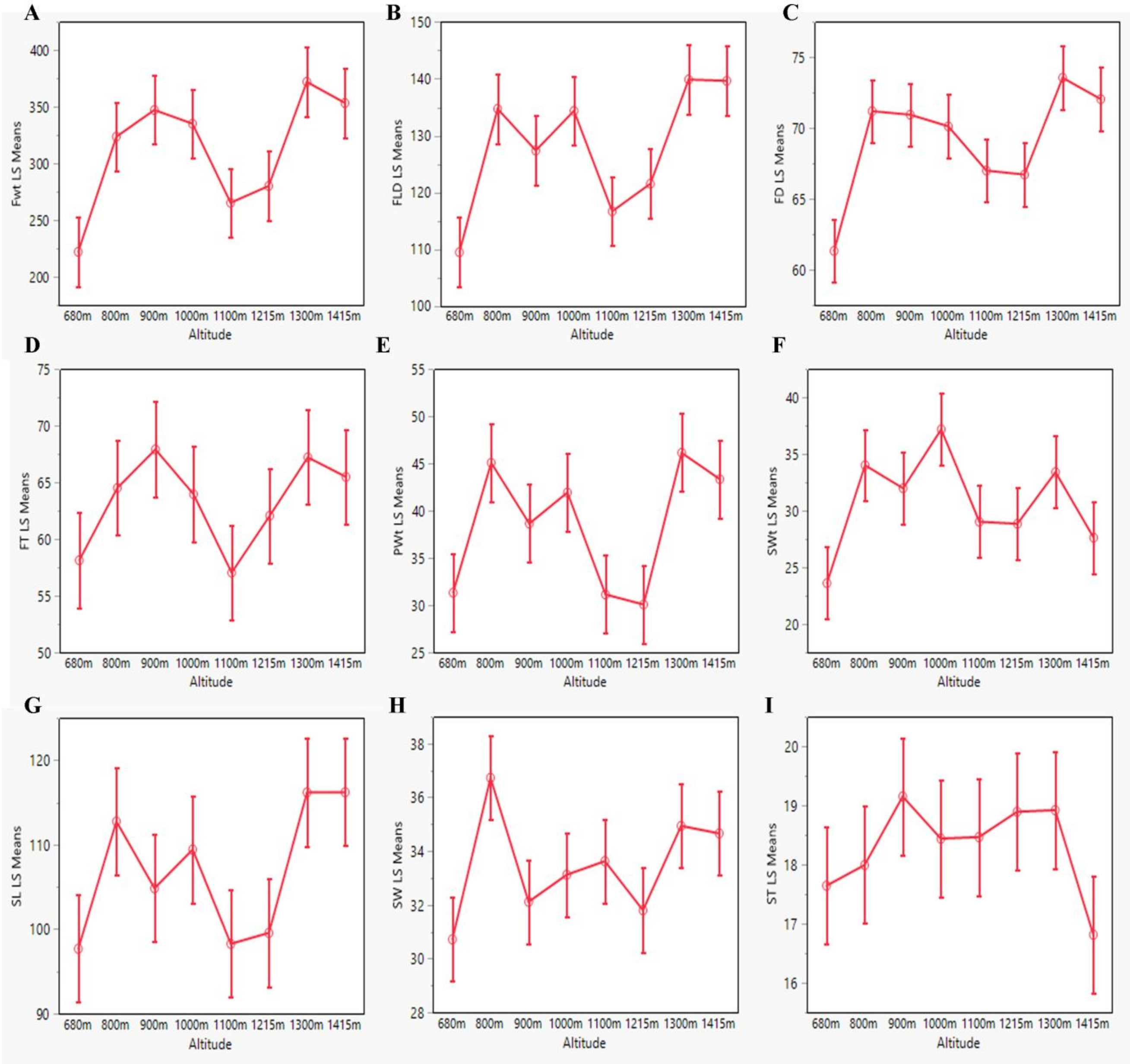

Figure 2. The least-square means for morphological traits: (**A**) Fruit weight (Fwt); (**B**) Fruit longitudinal diameter (FLD); (**C**) Fruit transversal diameter (FD); (**D**) Fruit thickness (FT); (**E**) Peel weight (Pwt); (**F**) Seed weight (Swt); (**G**) Seed length (SL); (**H**) Seed width (SW); and (**I**) Seed thickness (ST).

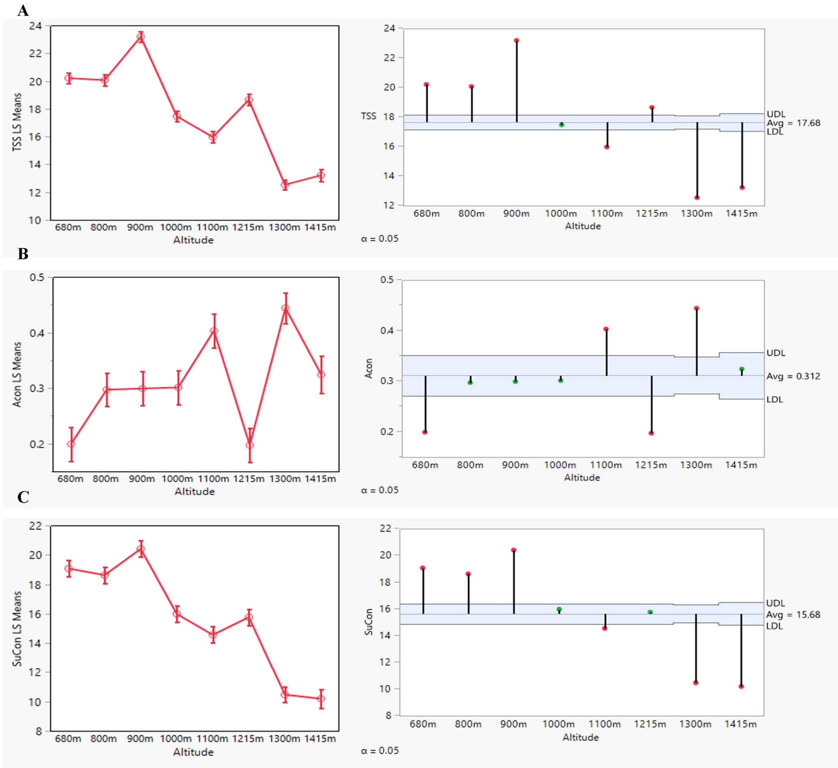

Figure 3. The least-square means for quality parameters (left: least square means; right: comparison with average), including: (**A**) Total soluble solids (TSS); (**B**) Total acid (Acon LS); and (**C**) Total sugar (SuCon).

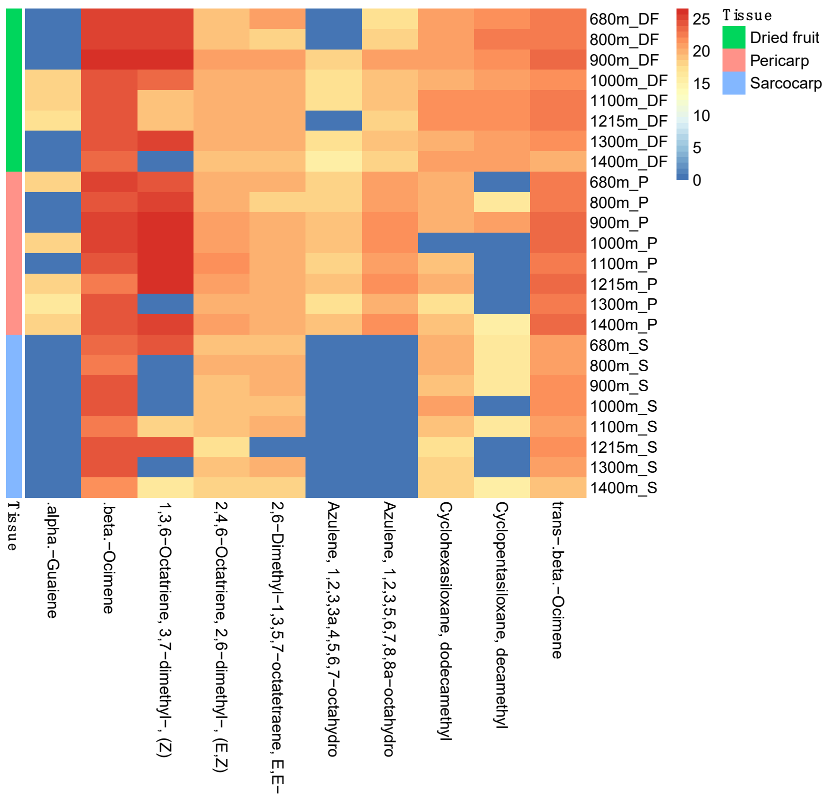

Figure 4. Heatmap showing changes in the accumulation of the individual volatile compound in the tissue of samples collected at different altitudes. Where DF = dried fruit; *p* = pericarp; and S = sarcocarp.

4. Discussion

Mango, with its worldwide consumption, is an economically important fruit and China ranks second in mango production worldwide [35]. In terms of fluctuating temperatures and abrupt rainfalls, climate change affects mango production in tropics, as in other crops [36–41]. In anticipation of increased temperatures, mango production in China is being shifted to higher altitudes [42]. The recent shift of mango production at higher altitudes to prevent the potential impact of climate change on mango production raised several queries concerning the impact of increased elevation on mango yield and fruit quality. Halder et al. [40] discussed the impact of increasing temperatures and CO_2 levels on the morpho-physiological characteristics of mango, including vegetative growth, flowering, photosynthesis, and fruit quality. Therefore, it is necessary to characterize mango cultivars for quality and yield at higher altitudes.

In China, Yunnan province is the production hub for several horticulture crops, including mango [6,43,44]. We used the established gardens of 'Myahintha' at different altitudes to assess the impact of altering altitudes on the yield and quality parameters and estimated significant differences across eight altitudes ranging from 680 to 1400 msl. We estimated a gradual increase in morphological characteristics, such as Fwt, FLD, FD, and FT, up to 1000 m elevation, and then a decrease above 1000 m. This shift in trend might occur due to environmental differences such as temperature, soil type, light intensity, and humidity at higher altitudes. Laurent et al. [45] described the impact of increased light intensity on photosynthesis, ultimately affecting fruit size in mango. Similarly, increased CO_2 concentration has been reported to improve fruit size in fruiting plants [46]. Moreover, we identified decreasing trend in quality parameters such as total soluble solids, total acid, and total sugar contents with the increase in altitudes. The decrease in quality parameters might be attributed to the early ripening due to favorable conditions for early maturity. Several studies have reported an increased sugar contents and soluble solids during slow ripening in different fruits [47–50]. The quality parameters depicted an opposite trend compared to morphological characteristics. However, further study with multiple genotypes across multiple altitudes is required to fully understand the impact of altering altitude on quality parameters.

Moreover, we characterized alteration in volatile compounds in dried fruit, pericarp, and sarcocarp, and identified 110 volatile compounds with different accumulation patterns at different altitudes. A diverse array of volatiles contributes to the overall flavor of the fruit and is affected by several factors, either pre-harvest or post-harvest [51]. Several genetic, environmental, cultural, and developmental factors influence fruit quality [52–55]. We further identified ten volatile compounds with the most frequent differential occurrence at different altitudes. Alpha-Guaiene is known for its significant role in flavor development in various plants [56–59]. In dried fruit, it was only identified at 1000–1215 m altitudes, suggesting that pre-harvest environmental factors might have influenced its accumulation pattern at different altitudes. Moreover, it was identified in pericarp tissues at 680 m, 1000 m, 1215 m, and above. With the available data, it is difficult to assess the actual factors behind this differential accumulation pattern.

Similarly, Beta-Ocimene and trans-beta-Ocimene were also identified with differential accumulation patterns in three tissues across all altitudes, with the highest accumulation of Beta-Ocimene at 900 m elevation in dried fruit and pericarp. Beta-Ocimene has been previously identified in several fruits [60–63]. Lapsongphol et al. detected a differential accumulation pattern of Beta-Ocimene in dried longan fruit at different temperatures [64]. Similarly, Janzantti et al. depicted a differential accumulation of Beta-Ocimene in passion fruit at different ripening stages [63]. Jaleel et al. identified Cyclohexasiloxane dodecamethyl and other volatile compounds in mango fruit [65]. Based on previously published reports, it is assumed that both pre-harvest and post-harvest factors can influence the accumulation pattern of volatile compounds in mango fruit. Moreover, we also identified several volatile compounds with the less frequent occurrence at different altitudes in three tissues. The accumulation pattern of volatiles suggested a considerable influence of environmental factors associated with altering altitudes. However, there was no clear trend of volatile accumulation at different altitudes due to the weak dataset (one year). A multi-year and muti-environment assessment is required to fully grasp the environmental impact on mango fruit and its quality.

To our knowledge, this is the first study investigating the effect of altering altitudes on the quality and yield attributes of mango fruit. Preliminary findings suggested a significant influence of altering altitudes on morphophysiological traits. However, further study is required to examine genotype–environment interactions with multiple genotypes to broaden the scope of environmental effects on mango yield and quality.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14100876/s1, Figure S1. Comparison of morphological traits with the overall average of each trait; Table S1. Factor loading for principal component analysis; Table S2. Summary of volatile compounds identified in different tissues and different altitudes; Table S3. Frequently accumulated volatile compounds in dried fruit, Pericarp, and Sarcocarp tissues at different altitudes.

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