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Multivariate Analysis of the Phenological Stages, Yield, Bioactive Components, and Antioxidant Capacity Effects in Two Mulberry Cultivars under Different Cultivation Modes

Na Zhang [1,2,3,4], Jinxin Li [5], Changyu Qiu [1,2,3], Wei Wei [1,2,3], Sheng Huang [1,2,3], Yong Li [4], Wen Deng [4], Rongli Mo [1,2,3,4,*] and Qiang Lin [1,2,3,*]

1 Sericulture Technology Extension Station of Guangxi Zhuang Autonomous Region, Nanning 530007, China; zhangnayuanwu@126.com (N.Z.); changyuqiu2008@163.com (C.Q.); gxcanyeweiwei@126.com (W.W.); gxcyhs@163.com (S.H.)
2 Key Laboratory of Genetic Improvement and Efficient Breeding of Silkworm and Mulberry, Guangxi Zhuang Autonomous Region, Nanning 530007, China
3 Research Academy of Sericultural Science of Guangxi Zhuang Autonomous Region, Nanning 530007, China
4 Institute of Economic Crops, Hubei Academy of Agricultural Sciences, Wuhan 430064, China; liyong8057@hbaas.com (Y.L.); dengwen@hbaas.ac.cn (W.D.)
5 College of Horticulture and Forestry, Yangtze University, Jingzhou 434025, China; lijinxinfly@163.com
* Correspondence: moqianchun1987@163.com (R.M.); gxlq67@163.com (Q.L.); Tel.: +86-0771-3277576 (Q.L.)

Abstract: Mulberry fruits are rich in bioactive components renowned for their antioxidant properties and potential health benefits. This study thoroughly investigated the impact of cultivation modes on the phenological stages, yield, bioactive components, and antioxidant activity of two mulberry cultivars, Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS). Notably, greenhouse cultivation led to earlier phenology and shorter fruit development maturation durations compared to field cultivation. Despite a decrease in fruit production and firmness, the greenhouse-grown mulberries exhibited higher individual fruit fresh weight. The content of bioactive components, encompassing anthocyanins, polyphenols, flavonoids, and vitamin C, and of antioxidant activity (measured in the FRAP and DPPH radical scavenging assays) was found to be lower in the greenhouse-grown mulberries than in those cultivated in the field. The contents of total polyphenols and flavonoids showed robust positive correlations in the FRAP and DPPH radical scavenging assays, which suggests that the antioxidant activity of mulberry fruit might be primarily attributable to the bioactive components of total polyphenols and flavonoids. Interestingly, the sugar content and hydroxyl radical scavenging activity (HRSA) displayed an inverse relationship between the two cultivars in the greenhouse versus field conditions. The multivariate analysis highlighted distinct patterns for different cultivars under varying cultivation modes. This study underscores the potential to enhance bioactive components and antioxidant activity through effective manipulation of climate conditions, thereby unlocking the full nutritional potential of mulberry fruits on a large scale in greenhouse environments.

Keywords: mulberry; fruit quality; bioactive components; antioxidant capacity; cultivation modes

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1. Introduction

Within the contexts of a rural revitalization strategy and the increasing pursuit of human leisure, leisure agriculture has developed into an emerging avenue for rural economic growth and industrial upgrading [1]. This agriculture has been vigorously promoted by national governments as a rural industry [2]. As a critical portion of the leisure agriculture industry [3], picking orchards are extensively used for various vegetables and fruits, including potatoes, cucumbers, mini-pumpkins, cherries, apples, grapes, and Chinese strawberries [1].

The mulberry is a multipurpose tree with a broad global distribution. Mulberry fruit is often noted for its richness in bioactive compounds and antioxidant activity, based on previous research [4], which has earned its notoriety as a "superfood" in European countries [5]. Consequently, the mulberry represents the optimal contemporary leisure and sightseeing tree species. However, the planting density and tree shape that are encouraged by conventional mulberry cultivation are unsuitable for picking tourism's rapid development [6]. Mulberry fruits in open fields can easily be infected by pathogens causing sclerotiniosis [7] and eaten by insects and birds, severely limiting marketable fruit yields. Some mulberry cultivars (including Taiwan changguosang and Baichangguo) are susceptible to the reduced temperatures experienced in late spring that cause fruit to drop. Additionally, the fruits are highly perishable following harvest and challenging to store and transport, further limiting the economic feasibility of planting mulberry trees for fruit production on a commercial scale [8]. Therefore, to enhance the value of mulberry fruit, it is critical to plant mulberry trees for fruit production in greenhouses.

Greenhouse cultivation is an effective and widely utilized technology that provides plants with optimally controlled microclimate growth conditions, extending the production season and protecting against diseases and insects. Greenhouses are predominantly used for off-season fruit and early spring fruit production [9–11]. Therefore, information on the agronomic characteristics of mulberry trees under greenhouse conditions is necessary for producers to understand the cultivars that are most ideally suited to their applications. While greenhouse cultivation has been investigated for its commercial value on black mulberry fruit over the past several years [12–15], a limited number of studies have focused on China's main cultivated mulberry species. Moreover, few studies have evaluated the characteristics of fruit yield and quality arising from different cultivation modes.

Therefore, this study aimed to systematically investigate and contrast the climate conditions, plant phenology, fruit yield, chemical composition, and biological activity of two primary cultivars (Yueshen Dashi and Xinjiang Baisang) growing under two different cultivation modes. Moreover, we sought to evaluate the prospects for application in high-value mulberry fruit in a greenhouse environment. Our findings provide insight into increasing the content of bioactive components and antioxidant activity through effectively controlling climate conditions and realizing the high-yield, stable-yield, high-quality, and standardized cultivation of mulberry at a large scale in a greenhouse. Multivariate analysis was used to investigate the contribution and correlation of variables for fruit yield and quality characteristics.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

In our study, two mulberry cultivars, Yueshen Dashi (YS-DS) (*Morus atropurpurea* Roxb.) and Xinjiang Baisang (XJ-BS) (*Morus alba* L.), were employed. The mulberry trees were grafted onto Guisangyou 12 (*Morus atropurpurea* Roxb.) rootstocks using pocket grafting in 2019. The plant material was maintained at the mulberry germplasm resource nursery of the Industrial Crops Institute of Hubei Academy of Agricultural Sciences situated in Wuhan City (central China, 30°48′7″ N, 114°33′4″ E).

We chose two different cultivation modes at the mulberry germplasm resource nursery. The experiment was composed of four treatments (two mulberry cultivars × two cultivation modes) with nine biological replicates per treatment (one tree representing one replicate). The greenhouses consisted of three identical adjacent double-span greenhouses. Each greenhouse was 48 m × 12 m, covered by a fogging-resistant polyethylene film with a planting area of 384 m^2. The orientation of the greenhouses was north–south. There was no heating system, and the interior temperature was maintained via natural ventilation using roof and side vents. To ensure the improved utilization of the greenhouse space and to enable sightseeing picking, the trunks of the YS-DS trees were extended horizontally and fixed on a wire in the same direction, while the side branches were trained upward into a Y shape rather than horizontally along a wire (Figure 1B). The XJ-BS trees in the

greenhouse were trained to a horizontal trellis at a height of 2 m (Figure 1C). Mulberry trees were spaced at 2 m × 4 m for the two cultivation modes, and the water and fertilizer management were identical.

Figure 1. Different cultivation modes for mulberry trees. (**A**) Field cultivation mode. (**B**) Y shape for YS-DS in the greenhouse. (**C**) Horizontal trellis structure for XJ-BS in the greenhouse.

2.2. Climate Factors

The meteorological data utilized in the field cultivation mode consisted of real-time weather data acquired using a small weather station (Dynamet-1k, Dynamax Inc., Houston, TX, USA). The meteorological factors within the greenhouse were recorded using three Temperature and Humidity Data Logger Recorders (GSP-6, Elitech Technology Co., Ltd., Hangzhou, China), and the sensors encompassed one air temperature and one humidity sensor (2.0 m above the ground with resolutions of 0.5 °C and 1%, respectively). All meteorological data for the two cultivation modes were automatically recorded at 60 min intervals. The data was recorded from November 2021 to October 2022, which included the entire phenological growth period of the mulberry trees.

2.3. Phenological Observation

In our previous study, the BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie) scale of mulberry was established via phenological observation over the course of two annual growing seasons (2020–2022) [16]. In this work, three growth stages of the YS-DS and XJ-BS cultivars under different cultivation methods, including the buds elongating and bursting (stage 02), full flowering (stage 55 where 50% of flowers were open), and fruit being ripe enough for picking (stage 87), were recorded throughout 2021–2022 using the BBCH scale.

2.4. Fruit Firmness and Yield

Fruits from similar locations in the trees were randomly obtained at the commercially ripe stage according to the BBCH scale. The date of fruit picking varied across mulberry cultivars and cultivation modes, as illustrated in Table 1. The firmness of the mulberry fruit was measured using a CT3 texture analyzer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) and expressed in terms of Newtons (N). The individual fruit fresh weight (FW, grams), fruit length (FL, millimeters), and fruit diameter (FD, millimeters) were logged using an electronic balance (ME203E, Mettler Toledo Technology Co., Ltd., Shanghai, China) and digital vernier calipers (3V Battery Digital Caliper, Guilin Guanglu Measuring Instrument Co., Ltd., Guilin, China). The total number of buds per plant (TNBs, number per plant) and the average fresh fruit number per plant (AFFN, number per plant) were assessed at the inflorescence emergence stage and the fruit setting stage, respectively. Additionally, the total production per plant (TP, kilograms per plant) was calculated by multiplying the TNBs by the AFFN and the FW and then dividing the total by 1000.

Table 1. Primary phenological growth stages of Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS) mulberry cultivars using different cultivation modes.

Cultivars	Cultivation Modes	Buds Elongating and Bursting	Full Flowering	Fruit Ripe for Picking
YS-DS	Greenhouse	17 January 2022	10 March 2022	15 April 2022
	Field	24 January 2022	15 March 2022	24 April 2022
XJ-BS	Greenhouse	28 February 2022	14 March 2022	1 May 2022
	Field	5 March 2022	27 March 2022	7 May 2022

2.5. Fruit Quality

2.5.1. Sugar and Acid Determination

The total soluble solids (TSS) and total titratable acidity (TTA) were determined based on previously described methods [17]. The soluble sugar content (SSC) was assessed using the anthrone reagent method [18], and the SSC/TTA ratio was estimated. The reducing sugar content (RSC) was determined using the dinitrosalicylic acid (DNS) reagent method using a previously described method [19].

2.5.2. Athocyanin and Vc Determination

As outlined in a prior study [20], the total anthocyanin (TA) of the mulberry fruits was measured using a pH differential method. Briefly, a 1 g sample was extracted using 10 mL of C_2H_5OH-HCl solution (85: 15 95% C_2H_5OH: 1.5 M HCl v/v) for 40 min (at 4 °C). The supernatant was diluted with the KCl-HCl solution (0.2 M, pH 1.0) and the NaAc-HAc solution (0.2 M, pH 4.5). The absorbance of the equilibrated reaction mixture solutions allowed to stand at room temperature for 40 min was measured using a UV-VIS spectrophotometer (UV-8000, Shanghai Metash Instruments Co., Ltd., Shanghai, China) at 510 nm and 700 nm. Distilled water was used as a blank. The results were expressed as mg of cyanidin-3-glucoside equivalent (CGE) per gram of fresh weight. The vitamin C (VC) level was measured using a xylene-2,6-dichlorophenol indophenol (DCPIP) colorimetry [21].

2.5.3. Preparation of Mulberry Fruit Extract

Fresh mulberry fruits were flash-frozen in liquid nitrogen and subsequently milled. An amount of 1 g of the sample was accurately weighed and 10 mL of precooled 80% methanol was added to the extract with ultrasound for 20 min. The supernatant was obtained by centrifugation at 10,000× g for 20 min at 4 °C and stored at −22 °C until analysis of the total polyphenols (TPO), total flavonoids (TF), and antioxidant activity.

2.5.4. Total Polyphenols and Flavonoids Determination

The TPO in the 80% methanol extract from the mulberry fruit was determined using the Folin–Ciocalteau colorimetric method [22]. The absorbance was measured at 760 nm. A calibration curve was prepared using gallic acid, and the results were expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight. The standard concentrations of GAE (1, 2, 4, 6, 8, 10, and 12 μg·mL^{-1}) were utilized to generate a calibration curve.

The TF was determined using the aluminum chloride ($AlCl_3$) colorimetric method with minor modifications [23]. Specifically, the 1.0 mL extract solution of 80% methanol was combined with 1.0 mL of 95% alcohol, 0.1 mL of 10% (w/v) $AlCl_3$, and 0.1 mL of potassium acetate (1 M). Subsequently, 2.8 mL of distilled water was also included in the mixture and was reacted at room temperature for 40 min. The absorbance at 415 nm was examined using a UV-VIS spectrophotometer (UV-8000, Shanghai Metash Instruments Co., Ltd., Shanghai, China). The total flavonoid content was expressed as the mg of quercetin (Sigma Chem. Co., St. Louis, MI, USA) equivalent (QE) per 100 g of fresh weight. Standard concentrations of quercetin (5, 10, 20, 40, 60, 80, and 100 μg·mL^{-1}) were used to generate a calibration curve.

2.5.5. Antioxidant Activity Analysis

The scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals was determined using a previously published method [24]. The final findings of the DPPH radical scavenging activity were computed and expressed as mg of Trolox equivalents (TE) per 100 g of fresh weight. Standard concentrations of Trolox (0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg·mL^{-1}) were employed in the preparation of a calibration curve. The final findings of the hydroxyl radical scavenging activity were determined and expressed as mg of L-ascorbic acid equivalents (AAE) per 100 g of fresh weight, and the standard concentrations of L-ascorbic acid (50, 100, 200, 400, 600, 800, 1000, and 1200 μg·mL^{-1}) were used to generate a calibration curve.

The ferric-reducing antioxidant power (FRAP) of mulberry fruit was determined using the spectrocolorimetric method described previously [24] with minor modifications to the preparation of the FRAP reagent. The FRAP reagent included a 0.3 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), and 20 mM ferric chloride (10:1:1, *v*/*v*/*v*) [25]. $FeSO_4$ standard solutions (100, 200, 400, 600, 800, 1000, 1200, and 1600 μM) were used to generate the calibration curves. The ferric-reducing ability of each sample was expressed as mmol of $FeSO_4$ equivalents per 100 g of fresh weight.

2.6. Statistical Analysis

Data were subjected to an analysis of variance (ANOVA), and differences among means were compared using the Duncan's multiple range test ($p < 0.05$) using SPSS software (version 26.0, IBM, Armonk, NY, USA). To create a hierarchically clustered heatmap and to show trait–trait correlations, a Pearson's correlation analysis and visualization was conducted using the 'seaborn.clustermap' python package (version 3.9.2) [26,27]. In addition, to classify mulberry cultivars derived from different cultivation modes in groups according to their horticultural potential, a principal component analysis (PCA) and linear discriminant analysis (LDA) were performed. The PCA was conducted using the '*FactoMineR*' (for data analysis) and '*factoextra*' (for data visualization) packages in *RStudio* (version 4.3.1) [28]. The LDA was performed using Python 3.9.2.

3. Results

3.1. Climate Factor Variation and Phenological Stages in Various Cultivation Modes

The trends in temperature and relative humidity in different cultivation modes were entirely consistent with the temperature and relative humidity remaining significantly higher within the greenhouse than in the field (Figure 2A–D). The mean annual temperatures of the greenhouse and the field from November 2021 to October 2022 were 20.13 °C and 18.41 °C, respectively. The mean annual maximum temperature in the greenhouse was 27.97 °C, whereas in the field it was approximately 22.17 °C (Figure 2E). The mean annual minimum temperatures were 15.18 °C and 14.79 °C in the greenhouse and the field, respectively (Figure 2E). Moreover, the annual mean, maximum, and minimum relative humidities in the greenhouse were 80.14%, 92.26%, and 61.02%, respectively, whereas in the field they were 60.87%, 77.62%, and 44.29%, respectively (Figure 2B,D). The monthly temperature variations between day and night in the greenhouse were also higher than in the field, even in May when the temperature difference was minimal (Figure 2E). Additionally, the daily mean temperature curves across the different cultivation models in the fruit ripening stage (April–May 2022) were essentially aligned, especially in May (Figure 2F).

The data from three primary phenological stages (bud bursting, flowering, and fruit maturity period) of two mulberry cultivars in the greenhouse and field were assessed, as shown in Table 1. It was observed that the phenological stages of the mulberry took place in the greenhouse 5–13 days earlier than in the field. Moreover, the greenhouse condition reduced the durations of fruit development and maturation for the two mulberry cultivars. The XJ-BS always had later budbreak, flowering, and fruit maturation than the YS-DS in the same cultivation conditions.

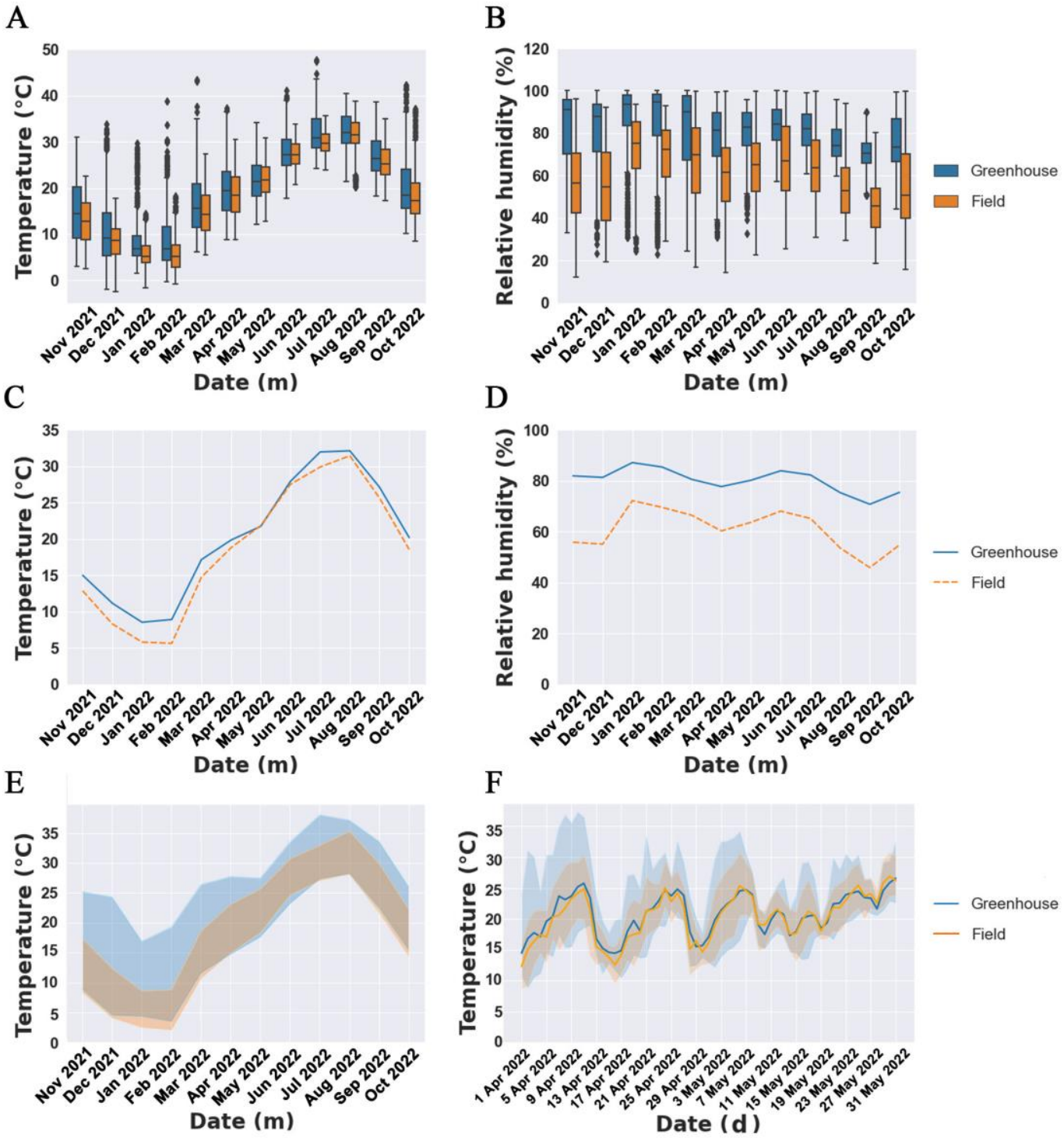

Figure 2. Climate factors in different cultivation modes. The dates include the period from November 2021 to October 2022. (**A**,**B**) Temperature and relative humidity variation across the two cultivation modes. Each box plot represents 720 replicates, with the inner line showing the median, the notches representing the bootstrapped 95% confidence interval of the median, the ends of the box representing the first and third quartiles, and the dots representing outliers. (**C**,**D**) Monthly average line chart of temperature and relative humidity. (**E**) Monthly temperature variations between day and night. Shades represent the temperature difference under different cultivation approaches. (**F**) Daily mean, maximum, and minimum temperatures in April and May 2022 under different cultivation approaches.

3.2. Fruit Firmness and Yield in Different Cultivation Modes

The firmness and fruit production were assessed over different cultivation modes (Table 2). Our findings demonstrated that the highest fruit firmness between the two mulberry cultivars was observed in the field, with no significant difference between the different cultivars using the same cultivation mode. The total production (TP) was associated with the TNBs, AFFN, and FW, and its value was primarily driven by the TNBs. Compared to the greenhouse, the TNBs in the field were significantly higher. Likewise, the TP in the field was significantly higher than in the greenhouse. For instance, the field- and greenhouse-grown YS-DS showed a mean TP of 10.99 kg plant^{-1} and 6.32 kg plant^{-1}, respectively, exhibiting a 1.7-fold increase in field yield. Furthermore, the fruit sizes of different genotypes had a differential response to the cultivation modes. The FW and FL of the XJ-BS were significantly higher in the greenhouse than in the field, but there was no significant difference found in the YS-DS.

Table 2. Fruit firmness, size, and production across Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS) mulberry cultivars using different cultivation modes.

Cultivars	Cultivation Modes	FW (Grams per Fruit)	FL (Millimeters per Fruit)	FD (Millimeters per Fruit)	Firmness (Newtons)	TNBs (Number per Plant)	AFFN (Number per Bud)	TP (Kilograms per Plant)
YS-DS	Greenhouse	3.83 ± 0.53 a	33.16 ± 1.48 a	14.37 ± 0.87 a	0.53 ± 0.01 b	276.44 ± 17.52 b	5.98 ± 0.45 a	6.32 ± 0.65 c
	Field	3.53 ± 0.49 a	33.14 ± 1.44 a	14.50 ± 0.66 a	0.64 ± 0.02 a	486.56 ± 82.49 a	6.39 ± 0.54 a	10.99 ± 2.14 a
XJ-BS	Greenhouse	3.59 ± 0.71 a	28.98 ± 1.21 b	14.12 ± 0.61 a	0.54 ± 0.01 b	250.11 ± 56.56 b	5.48 ± 0.26 c	4.92 ± 1.06 d
	Field	2.85 ± 0.59 b	24.93 ± 3.01 c	14.76 ± 1.91 a	0.65 ± 0.03 a	515.22 ± 69.97 a	5.55 ± 0.34 c	8.19 ± 1.42 b

Data are presented as mean ± standard deviation, using nine biological replicates. The different letter(s) in each column denote significant differences among means at $p < 0.05$ (Duncan test). FW (fruit fresh weight); FL (fruit length); FD (fruit diameter); TNBs (total number of buds); AFFN (average fresh fruit number); and TP (total production).

3.3. Fruit Quality across Different Cultivation Modes

Significant differences were identified in the quality of mulberry fruits when examined across cultivation modes (Figure 3). Likewise, significant differences were identified according to species. With respect to the sugar content, the YS-DS cultivars in the field had a higher SSC (11.12 g·100 g^{-1} FW) and RSC (10.60 g·100 g^{-1} FW) than those grown in the greenhouse, while the opposite occurred in the XJ-BS (Figure 3B,C). Compared to fruit produced in the field, the TTA of the greenhouse plants was increased significantly, while the ratio of SSC/TTA decreased significantly (Figure 3D,E). Moreover, the content of bioactive components (including the TA, TPO, Vc, and TF) was reduced in the greenhouse compared with plants from the field (Figure 3G–I,L). Similarly, the FRAP and DPPH radical scavenging activities of mulberry fruits in the greenhouse were significantly lower than mulberry fruits from the field (Figure 3J,K). Compared to other antioxidant activity measurements, the HRSA values for different mulberry cultivars were relatively homogenous. In this present study, the HRSA of different genotypes differed in response to different cultivation modes. The YS-DS from the field exhibited a greater HRSA (951.81 mg AAE·100 g^{-1} FW), which differed significantly from those grown in the greenhouse, while the opposite occurred in the XJ-BS (Figure 3F). Moreover, there was no significant difference in the TSS and VC of the YS-DS and the TA and TPO of the XJ-BS across different cultivation modes (Figure 3A,G–I).

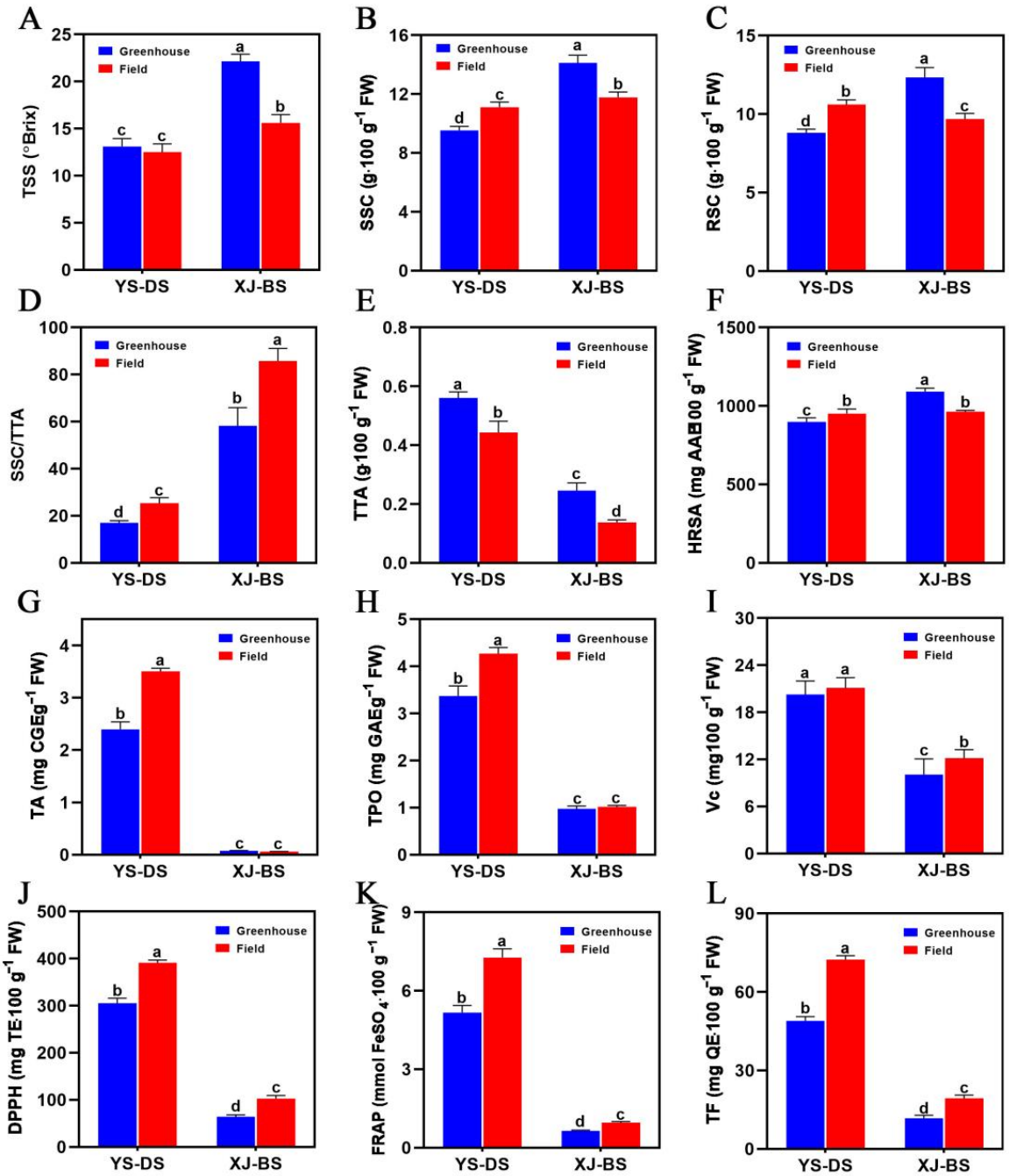

Figure 3. Phytochemical quality of Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS) mulberry grown using different cultivation modes. (**A**) Total soluble solid (°Brix). (**B**) Soluble sugar content (g·100 g^{-1} FW). (**C**) Reducing sugar content (g·100 g^{-1} FW). (**D**) Soluble sugar content/total titratable acidity. (**E**) Total titratable acidity (g·100 g^{-1} FW). (**F**) Hydroxyl radical scavenging activity (mg AAE·100 g^{-1} FW). (**G**) Total anthocyanin (mg CGE·g^{-1} FW). (**H**) Total polyphenols (mg GAE·g^{-1} FW). (**I**) Vitamin C (mg·100 g^{-1} FW). (**J**) DPPH radical scavenging activity (mg TE·100 g^{-1} FW). (**K**) Ferric reducing antioxidant power (mmol $FeSO_4$·100 g^{-1} FW). (**L**) Total flavonoids (mg QE·100 g^{-1} FW). Data are presented as mean ± standard deviation. Different letter(s) on the columns indicate a difference at a significance level of $p < 0.05$ (Duncan test).

3.4. Correlation Analysis of Fruit Yield and Quality Traits

The results of our correlation analysis indicated the presence of correlations among different fruit traits, revealing three clusters of linked traits (Figure 4). Cluster 1 was composed of the SSC/TTA, RSC, SSC, TSS, and HRSA, which were predominantly tied to

fruit taste. Within Cluster 1, the SSC and HRSA were most strongly correlated (r = 0.941, p = 0). The SSC and RSC were also highly correlated traits (r = 0.906, p = 0). Cluster 2 mainly comprised bioactive components and antioxidant activity, including the VC, TF, DPPH, FRAP, TA, TPO, and TF. The TPO and FRAP were the most heavily correlated traits within this cluster (r = 0.997, p = 0). In comparison to Cluster 1 (r mean = 0.707, variance = 0.056), Cluster 2 exhibited a higher correlation (r mean = 0.822, variance = 0.022) and lower variance. This result indicates that the traits in Cluster 2 were, on average, more correlated to one another than in Cluster 1. Cluster 3 comprised the TP, TNBs, firmness, and fruit size, which were tied to fruit yield.

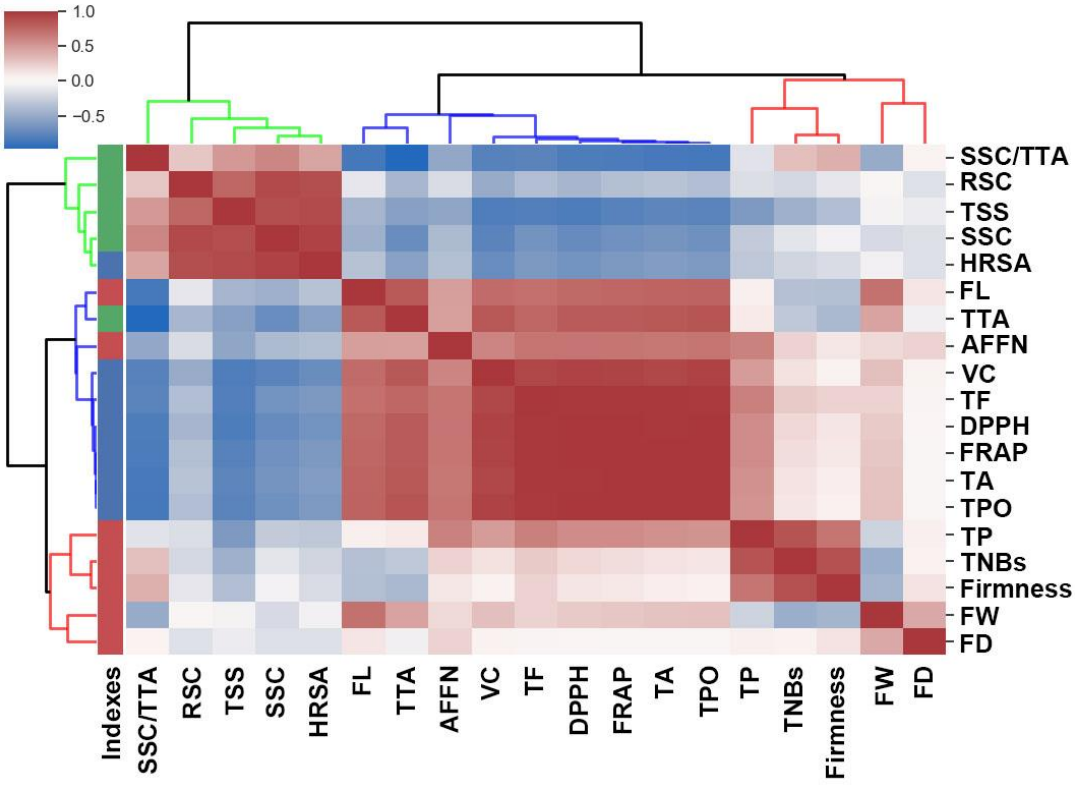

Figure 4. Cluster map of Spearman's rank correlation coefficient across traits. 'Average' linkage was employed for the hierarchical clustering. Traits were examined according to their correlation with other traits using *Seaborn Clustermap* in Python. Correlations of r = 1 are depicted on the diagonal as each trait correlates entirely with itself. Blue and red colors denote negative and positive correlation, respectively. Lighter colors indicate a low correlation of traits, while darker colors indicate correlations closer to 1 or −1. Clusters 1 and 2 are denoted in green and blue on the dendrogram, respectively, while Cluster 3 is colored red. FW (fruit weight); FL (fruit length); FD (fruit diameter); TNBs (total number of buds); AFFN (average fresh fruit number); TP (total production); TSS (total soluble solid); SSC (soluble sugar content); RSC (reducing sugar content); SSC/TTA (soluble sugar content/total titratable acidity); TTA (total titratable acidity); HRSA (hydroxyl radical scavenging activity); TA (total anthocyanin); TPO (total polyphenols); VC (vitamin C); DPPH (DPPH radical scavenging activity); FRAP (ferric reducing antioxidant power); and TF (total flavonoids).

3.5. Principal Component Analysis (PCA) of Fruit Yield and Quality Traits

The PCA model was used to assess the impact of different cultivation modes on the examined indices of the mulberry fruits. The cumulative contribution rate of the first four eigenvalues accounted for 93.13% of the total variability (Table 3). The PC1 was positively and strongly correlated with the content of bioactive components and antioxidant activity, including the VC, TF, DPPH, FRAP, TA, and TPO, but negatively associated with the TSS, SSC, RSC, SSC/TTA, and HRSA (Figure 5A). The PC2 was influenced by fruit firmness and yield (including the TNBs, TP, FW, and FL) (Figure 5A). In addition, the contribution of

the attributes was assessed, with the levels of the DPPH and TNBs exhibiting the greatest contribution to PC1 and PC2, respectively (Table 4). Replicates conducted with the same mulberry cultivars using different cultivation modes were clustered, but no overlap was observed among them. The same cultivation mode using different genotypes was also evidently allocated to the first two components (Figure 5B). These findings distinguished the microclimate of the two different cultivation modes across the two mulberry cultivars.

Table 3. Eigenvalues and cumulative contribution rates of each principal component.

Principal Component	Eigenvalues	Proportion of Variance	Cumulative Proportion
1	3.28	56.74	56.74
2	1.92	19.35	76.09
3	1.37	9.93	86.02
4	1.16	7.11	93.13

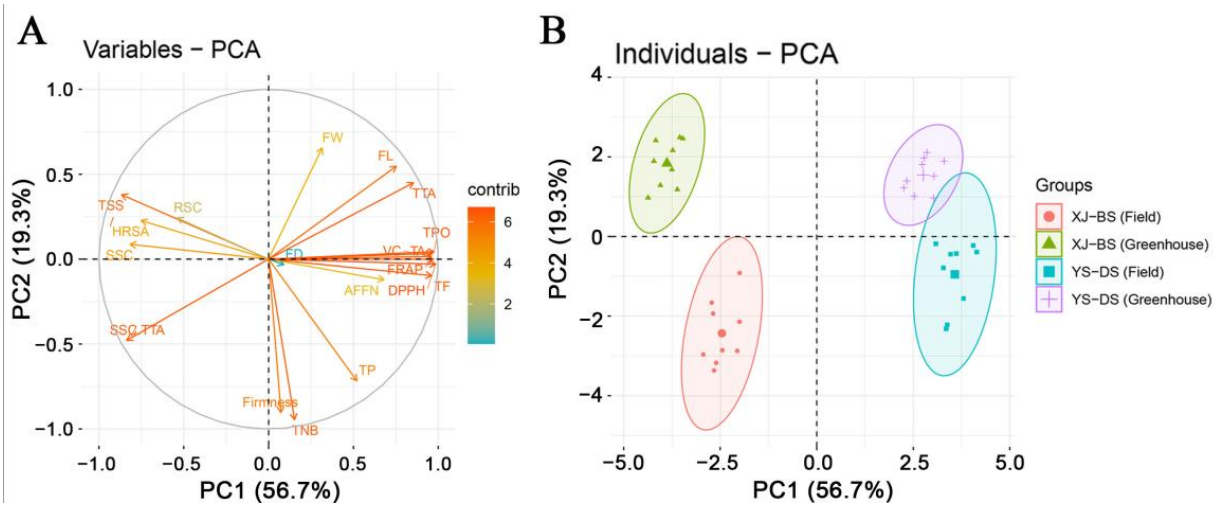

Figure 5. Principal component analysis (PCA) conducted on Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS) mulberry cultivars grown in the greenhouse and field. (**A**) Contribution percentage of fruit yield and quality. (**B**) PCA illustrating the clustering of the two mulberry cultivars across the different cultivation modes. FW (fruit fresh weight); FL (fruit length); FD (fruit diameter); TNBs (total number of buds); AFFN (average fresh fruit number); TP (total production); TSS (total soluble solid); SSC (soluble sugar content); RSC (reducing sugar content); SSC/TTA (soluble sugar content/total titratable acidity); TTA (total titratable acidity); HRSA (hydroxyl radical scavenging activity); TA (total anthocyanin); TPO (total polyphenols); VC (vitamin C); DPPH (DPPH radical scavenging activity); FRAP (ferric reducing antioxidant power); and TF (total flavonoids).

Table 4. Contribution (%) of different indices in relation to the first two principal components.

Indices	Principal Component One	Principal Component Two
TNBs	1.17	15.74
AFFN	5.21	2.02
TP	3.98	11.93
FW	2.42	10.90
FL	5.76	9.12
FD	0.68	0.54
Firmness	0.56	15.05
TSS	6.63	6.32
SSC	6.25	1.45
RSC	4.12	4.03

Table 4. *Cont.*

Indices	Principal Component One	Principal Component Two
SSC/TTA	6.41	7.97
TTA	6.54	7.49
HRSA	5.75	3.78
TA	7.39	0.55
TPO	7.46	0.73
VC	7.34	0.26
DPPH	7.54	0.49
FRAP	7.44	0.02
TF	7.36	1.62

FW (fruit fresh weight); FL (fruit length); FD (fruit diameter); TNBs (total number of buds); AFFN (average fresh fruit number); TP (total production); TSS (total soluble solid); SSC (soluble sugar content); RSC (reducing sugar content); SSC/TTA (soluble sugar content/total titratable acidity); TTA (total titratable acidity); HRSA (hydroxyl radical scavenging activity); TA (total anthocyanin); TPO (total polyphenols); VC (vitamin C content); DPPH (DPPH radical scavenging activity); FRAP (ferric reducing antioxidant power); and TF (total flavonoids).

3.6. Linear Discriminant Analysis (LDA)

An LDA model was employed for the discrimination of the samples within a 2D space (Figure 6). The first discriminant function (DF1) was the basic function for the classification of the samples, which explained 89.58% of the variance. The DF2 explained 8.21% of the total variance (therefore, the total variance explained by these two functions was 97.80%). In addition, all samples could be classified into four groups via the LDA. The result of the classification presented the dissimilarity of different cultivars under various cultivation modes, confirmed by the PCA (Figure 5).

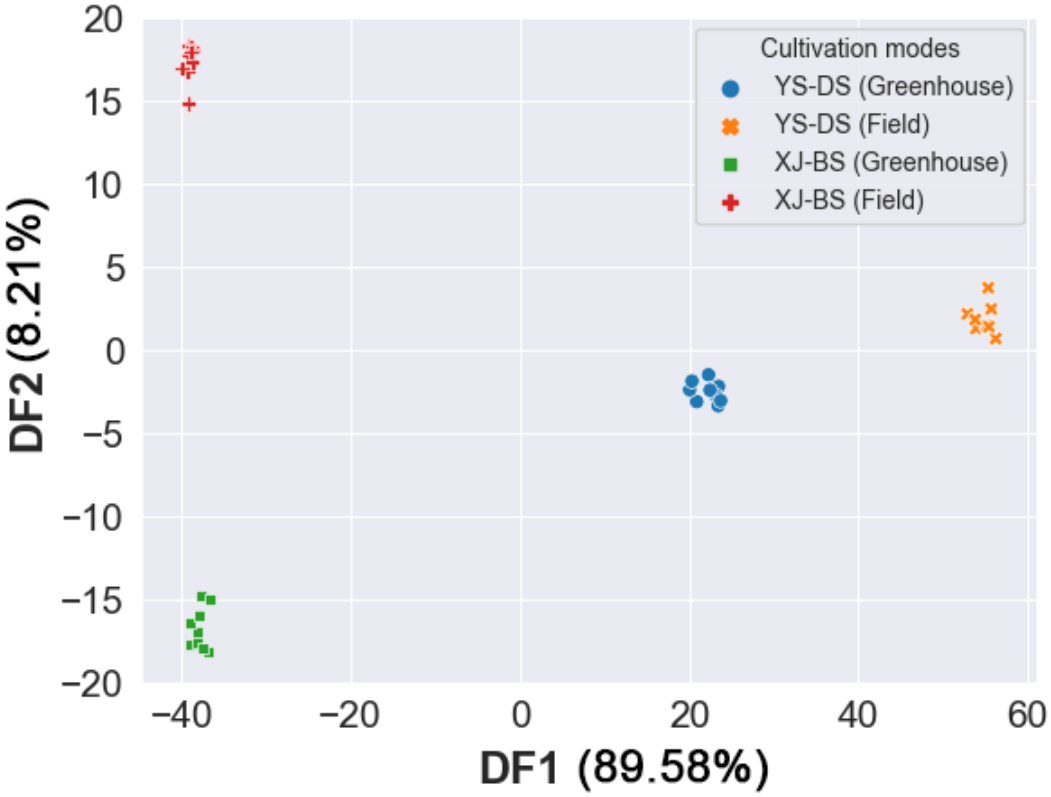

Figure 6. Linear discriminant analysis (LDA) plot for Yueshen Dashi (YS-DS) and Xinjiang Baisang (XJ-BS) mulberry cultivated in the greenhouse and field.

4. Discussion

While the impact of the cultivation environment is well-established with respect to its impact on fruit yield and quality, outside of a comparative study of different cultivation modes in black mulberry in Korea [14,15], no prior large-scale investigations have evaluated fruit production and quality under different cultivation modes in diverse mulberry types. In this current investigation, the yield and quality of mulberry fruit were influenced by both genotype and the environment. Generally, the field condition exhibited the optimal yield and quality of mulberry fruit compared to the greenhouse condition. The multivariate analysis demonstrated the presence of heterogeneous groups among cultivars and different cultivation modes. This was primarily due to the similarity in the content of bioactive components, the antioxidant activity, and the total number of buds per plant.

Phenology is critical for the implementation of management practices at specific stages of crop development (crop irrigation, fertilization, protection, pollination, harvesting, and pruning) that are predominantly impacted by the temperature. The phenological period of mulberry trees in the greenhouse was obviously earlier than those in the field, which is consistent with the findings of previous studies [12]. The differences identified in phenology in this study may be related to differences in temperatures. A previous study [29] determined that the mulberry bud dormancy release in early spring was modulated by low temperatures and long photoperiods. In this study, the greenhouse had elevated winter temperatures and shorter sunshine hours than the field. Therefore, we speculated that the mulberry trees in the greenhouse met the heat requirement earlier, causing early sprouting. Previous reports also confirmed our suspicions that, after meeting the chilling requirements, endodormant buds transform into an ectodormant state and can burst in response to growth-promoting factors, including warm temperatures [30–32]. Early flowering in greenhouses is also explained by the theory of "chilling and heat requirement", which is consistent with previous studies on sweet cherries [33].

Fruit yield under greenhouse conditions was reduced compared to field conditions. Compared to the field, the fruit yield of the YS-DS and XJ-BS cultivars that were grown in the greenhouse decreased by 42.5% and 40%, respectively (Table 2). Similar findings were reported in previous studies of berry yield using different growing conditions, such as with tomatoes [34] and grapes [35]. These results contrasted with other results [36,37], which reported the yield per tree of raspberries in a greenhouse was increased compared to fruits grown outdoors. Another study [38] reported that the total fruit yield was significantly impacted by shade, with increased shading leading to a reduction in the total fruit yield. Consistent with these reports, the differences in mulberry fruit yield may reflect variations in environmental and horticulture management, particularly with respect to the temperature, light, and pruning. Low light and high temperatures in greenhouses result in plant overgrowth. To control vegetative growth and to maintain tree shape, the greenhouse plants retained fewer branches after pruning, resulting in fewer TNBs per plant than in the field (Table 2). The AFFN linked to yield was unregulated by the environment but rather by genotype, and its values in the XJ-BS were greater than in the YS-DS (Table 2). Notably, the fruit was larger under greenhouse conditions, which was more attractive to consumers. This increase in size was possibly the result of the temperature, as previously reported [36]. At the same time, we identified that the texture of the mulberry fruit from the greenhouse was softer and not conducive to storage, but the taste of the berries was improved, which was tightly linked to the high temperature of the greenhouse [39]. Low light also reduced firmness, as reported previously [40] in 'Fuerte' and 'Hass' avocados exposed to direct sunlight, which exhibited firmer textures than fruits in shaded areas. In addition, the lack of seeds in the mulberry fruits in the greenhouse also contributed to their softer texture.

As the two primary metabolites of fresh fruits, sugar and organic acids are responsible for the sweetness and sourness of fruit, respectively [41]. Our study indicated that the TTA in greenhouse fruits was significantly elevated compared to those in the field, which coincided with findings displayed previously [15]. It is difficult to disentangle the effect of climatic variables on acidity, as the variables may impact plant physiological processes

at different stages of fruit growth [42]. However, there are many results suggesting that the TTA is reduced upon increased temperatures [43–46]. At low temperatures, the rate at which organic acids are synthesized exceeds their consumption rate in respiration [47]. These results were in line with our study finding that the average temperature in the field for six days prior to fruit ripening was higher than in the greenhouse (Figure 2F). For instance, the average temperature in the greenhouse (15–20 April) and field (19–24 April) for six days before the ripening of the YS-DS was 20.18 °C and 21.86 °C, respectively, and the daily average temperature clearly decreased and increased, respectively (Figure 2F). The average temperature at this stage was determined because the sixth day before the fruit ripening of the YS-DS was 30 days after flowering (in the greenhouse) and 33 days after flowering (in the field), respectively, when the TTA began to decrease rapidly as the fruit matured [48]. In addition, the TTA was also affected by light intensity. This was in line with a previous study [49], identifying that fruit harvested in the summer with high values for radiation had the lowest TTA values. A prior investigation [38] also found that shading increased the titratable acidity of fruits. The characteristic progression of mulberry ripening is the accumulation of sugar and the reduction of acid, and the ratio of SSC/TTA is a determining criterion for mulberry maturity. A previous report [14] documented that the free sugar content of mulberry was not impacted by the cultivation conditions. However, in 2015, a study found that the free sugar content in the greenhouse was elevated compared to the open field [15]. Our study demonstrated that different cultivation modes had differential effects on the SSC and RSC of the YS-DS and XJ-BS. Thus, these findings suggest that the genetic background determined the response of sugar content to climatic conditions. In addition, the sugar content was also impacted by various environmental aspects, such as light intensity, sunshine level, temperature, and humidity.

Exploratory studies of mulberry fruit have examined different health-promoting components such as vitamin C, anthocyanin, polyphenols, and flavonoids [50]. This present study demonstrated that the VC, TA, TF, TPO, FRAP, and DPPH radical scavenging activities in mulberry fruits derived from the field were elevated compared to the greenhouse, except the HRSA. A strong positive correlation between these indicators was observed. Similar results were reported previously [14,15], and the contents of polyphenols, anthocyanins, and flavonoids of mulberry grown in an open field were documented to be higher than those planted in a greenhouse. The increase in the content of bioactive components in the field can be explained by the increased light intensity and by a pronounced delay in fruit maturation. Anthocyanins are the largest class of flavonoids that provide benefits to human health and protect the plant from both light and oxidative stress [51]. Previous studies have documented that light was the most important environmental factor influencing anthocyanin biosynthesis in plants, and higher light intensity enhanced red coloration through the positive regulation of structural genes and regulatory gene expression in the anthocyanin biosynthesis pathway [52–55]. In our study, due to higher light intensity and increased fruit ripening time, the TA of YS-DS in the field was significantly higher than in the greenhouse. However, there was no significant difference in the TA of XJ-BS in different cultivation modes, perhaps because the cultivars with a low content of anthocyanin were not sensitive to variations of light intensity. Similar to anthocyanins, light intensity had a positive impact on the flavonoid content of fruits [54], which is consistent with our results. Flavonoids were the main phenolic compound in mulberry fruits [56,57]. Our findings demonstrated a strong correlation between the TPO and TF (r = 0.984, p = 0) (Figure 3). Additionally, the TPO was also highly correlated with DPPH (r = 0.993, p = 0) and FRAP (r = 0.997, p = 0), respectively (Figure 3), which is similar to previous reports [58,59]. These results indicate that the polyphenolic components of the extracts may play an important role in radical neutralization and lipid peroxidation inhibition. Furthermore, the DPPH free radical scavenging effects and the FRAP of mulberry fruits in the greenhouse were significantly reduced compared to the field. This was consistent with findings reported previously [60], in which the antioxidant activity was higher in tomatoes produced in an open field compared to in a greenhouse. Although the VC, TA, TF, TPO, FRAP, and

DPPH radical scavenging activities of mulberry fruits in the greenhouse were lower than those in the field, they remained elevated and did not significantly impact the health and medicinal value of the fruits. Hydroxyl radicals are relatively easily generated and possess mutagenic and carcinogenic effects due to their interactions with DNA [61]. Our results demonstrated that various cultivation modes had differing effects on the HRSA of the YS-DS and XJ-BS cultivars. Moreover, the HRSA was significantly positively correlated with the SSC ($r = 0.941$, $p = 0$), but not with the bioactive components, including polyphenols, anthocyanins, and total flavonoids (Figure 3). A previous study [62] reported that the HRSA may be related to the number of active hydroxyl groups in the molecule. The polysaccharides of mulberry fruits exhibited effective scavenging activity against hydroxyl radicals [63]. Hence, this may be explained by the extract with higher SSC containing higher polysaccharide content, resulting in more active hydroxyl groups. It can be observed that the XJ-BS with high hydroxyl radical scavenging activity also exhibits broad application potential in medicine, agriculture, and food.

5. Conclusions

In our study, we examined the phenology, fruit production, and quality of two mulberry species grown using different cultivation modes (in the greenhouse and the field). We suggest that the phenology of mulberry in the greenhouse was earlier than in the field, and the durations of fruit development and maturation in the greenhouse were shorter. Moreover, it was concluded that the field condition elicited much better performance in terms of yield, firmness, bioactive component content, and antioxidant activity (except the HRSA) in mulberry fruit compared to the greenhouse condition. The SSC, RSC, and HRSA of different genotypes had different responses to the two cultivation modes. Among the two mulberry cultivars, the YS-DS remains a satisfactory option for commercial cultivation due to its higher content of bioactive components, stronger DPPH free radical scavenging effects, and FRAP. Because of its higher SSC, RSA, and HRSA, the XJ-BS also has broad application potential. Moreover, multivariate analysis indicated that there were heterogeneous groups throughout cultivars and among cultivation modes, which confirmed that different cultivation modes had significant impacts on the yield and fruit quality of different mulberry genotypes. Proper use of a cultivation environment with appropriate light intensity, humidity, and ventilation should be considered to obtain robust outcomes. Hence, this study provides a foundational reference for producing high-value mulberry fruit in a greenhouse setting.

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