

## Influence of eight rootstocks on fruit quality of *Morus multicaulis* cv. 'Zijing' and the comprehensive evaluation of fruit quality traits

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### Abstract

Mulberry (*Morus L.*) has become an important crop throughout the world due to its fruits have been industrially exploited for various commercially valuable products. Many studies on mulberry related to genetic diversity, fruit quality, and breeding programs have been carried out, but little information on mulberry rootstocks is available, especially the possibility of applying grafting to improve the fruit quality. Here, we evaluated the effects of 8 different rootstocks on the fruit quality of 'Zijing' mulberry. Twelve fruit quality traits were extremely different except for the fruit shape index (FSI). 'Zijing' on 'Zheza 2' had the highest fruit weight (FW) and size, as well as titratable acidity (TA), but lower levels of other compounds content except the total soluble solids content (TSS) were detected. 'Yuesang 51' exhibited the highest soluble sugar content (SSC), reducing sugar content (RSC), SSC/TA ratio, anthocyanin content (AC) and the lower TA. In contrast, the lowest TSS, SSC and RSC were shown in 'Guisang 5'. Moreover, 'Guisang 12' exhibited the highest TSS and soluble protein content (SPC). The highest vitamin C content (VC) was observed in 'Guisang 6'. 'Tang 10 × Lun109', Zhenzhubai seedlings, 'Yuesang 11' together with 'Yuesang 51' had the lowest and similar levels of TA. Most importantly, these fruit quality traits were evaluated by principal component analysis (PCA), and 'Yuesang 51' with good comprehensive fruit quality was screened out, followed by 'Guisangyou 12'. Overall, these results contribute to evaluating the roles of different rootstocks on improving fruit quality of mulberry.

**Keywords:** correlation analysis; fruit quality; mulberry; principal component analysis; rootstock

### Introduction

In China, mulberry (*Morus L.*; Moraceae) is called "Sang Shen" and mainly grown for sericulture. Besides using the leaves, mulberry bears sweet fruit with various nutrients for human body (Jelled *et al.*, 2017; Pawłowska *et al.*, 2008; Huang *et al.*, 2017), which can be eaten fresh, or made for wine, tea, fruit juice, jam (Singhal *et al.*, 2010). In China, Korea and Japan, mulberry fruit can also be used in the pharmaceuticals industry for its various medicinal properties, and was designated as one of the first medicinal-and-edible plants

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by the Ministry of Health of China in 1985. It breaks down only purposes as syrup or adding flavors and natural colour in medicines (Singhal *et al.*, 2001, 2003). These applications accelerate the development of the mulberry fruit industry, and open a new vista for utilization of mulberry. Meanwhile, it poses new challenges to the quantity and quality of mulberry fruit seedlings, especially grafted seedlings.

As an important part of grafted seedlings, rootstock plays an important role in promoting their culture on different climatic and soil conditions and improving plant disease resistance (Dichio *et al.*, 2004; Cinelli and Loreti, 2004). These adaptive traits are conducive to improving fruit yields and quality (Webster, 1995, 2001; Nimbolkar *et al.*, 2016). Recently, the use of rootstocks were demonstrated to be an effective strategy in improving fruit yields and quality of cherry (Milošević *et al.*, 2020; López-Ortega *et al.*, 2016), plum (Popara *et al.*, 2020), sweet orange (Continella *et al.*, 2018), pear (Ikinci *et al.*, 2014), peach (Orazem *et al.*, 2011; Giorgi *et al.*, 2005), as well as vegetables fruit of tomato (Gioia *et al.*, 2010; Flores *et al.*, 2010; Turhan *et al.*, 2011), eggplant (Mozafarian *et al.*, 2020) and watermelon (Fallik and Ziv, 2020). Previous studies reported that many physiological and biochemical parameters such as fruit weight, fruit diameter, total soluble solids content, soluble sugar content, reducing sugar content, titratable acidity, soluble sugar content/titratable acidity ratio, anthocyanin content, etc. were measured to evaluate the fruit quality of different rootstock-scion combinations in lemon (Ali *et al.*, 2005), 'pioneer' Japanese plums (Daza *et al.*, 2008), sweet cherry (Gregorio *et al.*, 2016) and sweet orange (Continella *et al.*, 2018).

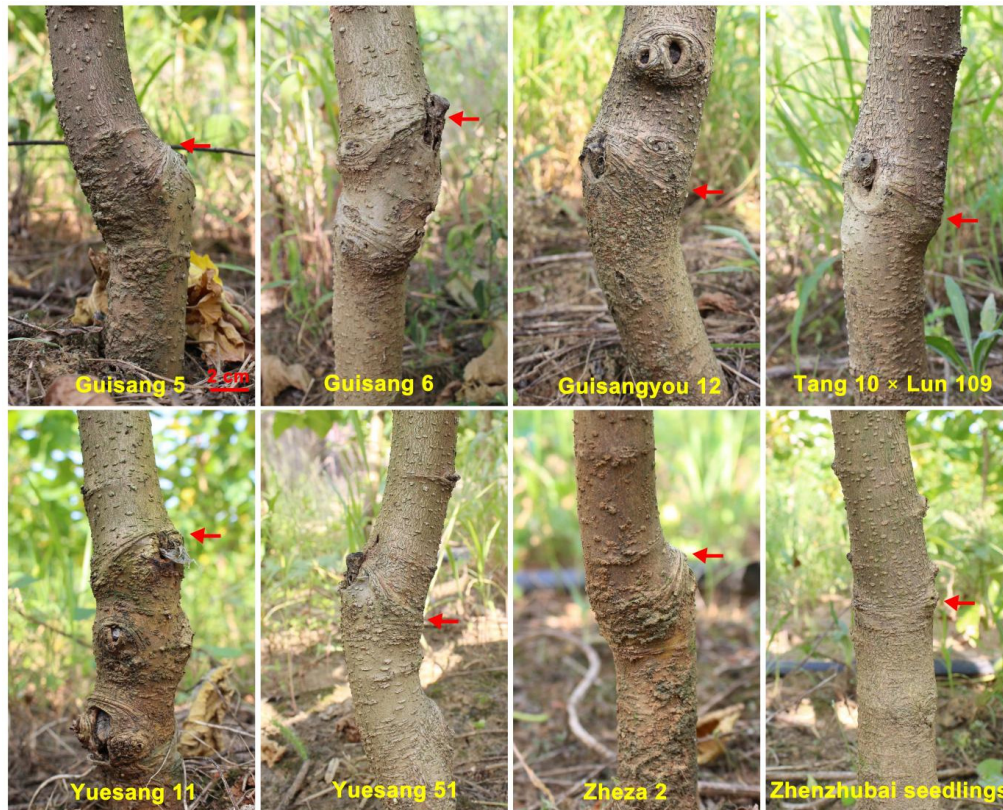
At present, the research on mulberry rootstocks mainly focuses on tolerance of drought, waterlogging and salt. Zhang *et al.* (2018) reported that grafting using Qinglong mulberry with salt tolerance as rootstock and Tieba mulberry with high yield and good quality as scion could improve salt tolerance. In addition, our previous study (Mo *et al.*, 2021) showed that the *Morus multicaulis* Perr. seedling has stronger drought tolerance, while *Morus atropurpurea* Roxb. seedling has stronger waterlogging tolerance. To our knowledge, the possibility of applying rootstocks to improve mulberry fruit quality has rarely been investigated. Therefore, the objective of the present study was to investigate the effects of different rootstocks on fruit quality of 'Zijing', a new mulberry cultivar with good taste and seedless. It is essential to the mulberry industry, and it will become a useful approach in commercial cultivation of mulberry fruit by using good rootstocks.

## Materials and Methods

### *Plant material*

The experiments were conducted at the experimental field for fruit growing of the Mulberry Repository of Hubei Province (latitude: 30.4481447, longitude: 114.327689 and altitude: 28 m), Wuhan, China. Eight rootstocks, including 'Guisang 5', 'Guisang 6', 'Guisangyou 12', 'Tang 10 × Lun 109', 'Yuesang 11', 'Yuesang 51', 'Zheza 2' and Zhenzhubai seedlings, were evaluated in grafting combinations with 'Zijing' [*M. multicaulis* Perr. 'Zhushan 3' (2X) × *M. atropurpurea* Roxb. 'Yueyou 78' (4X)], a fresh purple-colored mulberry cultivar selected by Institute of Economic Crops, Hubei Academy of Agricultural Sciences.

These seedlings were budded with 'Zijing' in July 2019, and all rootstocks were compatible with the scion (Figure 1). After surviving, budded plants were transplanted at a spacing of 0.5 × 2.0 m. The trial was design with 12 trees for each rootstock-scion combination, and they were randomly divided into 3 groups, each of which was a biological replicate. Measurements were carried out in the third year (2021) after planting.



**Figure 1.** The healing investigation of graft interface in different rootstocks in combination with ‘Zijing’ in third years after grafting on ‘Guisang 5’, ‘Guisang 6’, ‘Guisangyou 12’, ‘Tang 10 × Lun 109’, ‘Yuesang 11’, ‘Yuesang 51’, ‘Zheza 2’ and Zhenzhubai seedlings, respectively. Red arrows indicate graft interface. Scale bar = 2 cm

#### *Physical parameters*

In harvest season of 2021, mature fruits were manually and randomly collected according to the color and sense organs. A total of 150 fruits per rootstock-scion combination were selected from 12 trees. Fruit weight (FW, g) were recorded as the mean weight using an electronic balance (JE2001, Shanghai Puchun Measure Instrument CO., LTD, Shanghai, China). Fruit length (FL, mm) and diameter (FD, mm) were measured within 0.01 mm accuracy using a digital vernier caliper (3V Battery Digital Caliper, Guilin Guanglu Measuring Instrument CO., LTD, Guilin, China). Fruit shape index (FSI) was calculated based on FL/FD.

#### *Biochemical parameters*

Total soluble solid content (TSS, °Brix) of mulberry fruit juice was determined with a digital refractometer (PAL-1, Atago, Tokyo, Japan). The measurement range, resolution and accuracy of the instrument were 0.0-53.0 °Brix, 0.1% °Brix and  $\pm 0.2\%$  °Brix, respectively. The percent of titratable acidity (TA, expressed as the percentage of citric) was determined with fruit homogenized with water (add 1 g fruit sample to 100 ml with water) by titration using 0.05 N NaOH and 1% phenolphthalein indicator. Soluble sugar content (SSC, %) was determined by the anthrone reagent method according to the description of Morris (1948), and then SSC/TA ratio was estimated. The reducing sugar content (RSC, %) was measured using the dinitrosalicylic acid reagent method (Miller, 1959). All the above biochemical parameters were determined in three replicates.

*Bioactive compounds*

The soluble protein content (SPC, mg·g<sup>-1</sup>) was determined using the coomassie brilliant blue G-250 method as described in the study of Sun (2000). The VC (mg·g<sup>-1</sup>) of mulberry fruit was measured using Vitamin C assay kit (Colorimetric method) according to the instructions of Vitamin C assay kit (Colorimetric method, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The anthocyanin content (AC, nmol·g<sup>-1</sup>) of fruit was measured according to the method described in (Rabino and Mancinelli, 1986). All measurements were performed in three replicates.

*Statistical analysis*

Analysis of variance (ANOVA) was performed by Excel, and the numeric data was expressed as means ± S.D. SPSS software (Version 26.0, IBM, USA) was used for multiple range significant difference (Duncan) test ( $p < 0.05$ ) and partial correlation analysis, as well as principal component analysis (PCA). Sample parameters were converted to standardized values by using subordinate function (Tao, 1982; Xie and Liu, 2013), the equation is following:

$$U_{ij} = (X_{ij} - X_{j\min}) / (X_{j\max} - X_{j\min}) \quad (1)$$

Where  $X_{ij}$  ( $i = 1, 2, \dots, n$ ;  $j = 1, 2, \dots, p$ ) is the value of the  $i$ th rootstock and the  $j$ th fruit quality indicator,  $X_{j\min}$  is the minimum of the  $j$ th indicator of fruit quality and  $X_{j\max}$  is the maximum of the  $j$ th indicator.

The values of each integrated indicator of fruit quality is defined in the following equation (Wang and Xing, 2017):

$$\begin{aligned} Q_1 &= S_{11}U_1 + S_{12}U_2 + \dots + S_{1p}U_p \\ Q_2 &= S_{21}U_1 + S_{22}U_2 + \dots + S_{2p}U_p \\ &\dots\dots \\ Q_t &= S_{t1}U_1 + S_{t2}U_2 + \dots + S_{tp}U_p \end{aligned} \quad (2)$$

Where  $S_{1i}, S_{2i}, \dots, S_{ti}$  ( $i = 1, 2, \dots, t$ ) are the loading factor of principal component ( $S_{tp} = A_{tp} / \sqrt{\lambda_t}$ ,  $A_{tp}$  is the loading factor of component,  $\lambda_t$  is the eigenvalues) and  $U_1, U_2, \dots, U_p$  are the standardized values.

The comprehensive evaluation index is determined based on the following equation (Wang and Xing, 2017):

$$Q = Cr_1Q_1 + Cr_2Q_2 + \dots + Cr_tQ_t \quad (3)$$

Where  $Cr_1, Cr_2, \dots, Cr_t$  are the variance contribution rate corresponding to the principal components. A higher comprehensive evaluation index indicates a better quality of fruits.

**Results and Discussion***Physical properties of mulberry fruit*

The different rootstock significantly affected fruit physical attributes of mulberry ‘Zijing’ (Table 1). The fruit weights of 8 mulberry combinations ranged from 2.53 g to 3.06 g with an average of 2.70 g. Of these, ‘Zheza 2’ had the biggest fruits ( $3.06 \pm 0.19$  g), which was significantly larger than other rootstocks. The range of fruit weights was close to 2.54-3.02 g, which was reported in the four different mulberry cultivars from Pakistan (Iqbal *et al.*, 2010), and some indigenous mulberry genotypes from Turkish (Balik *et al.*, 2019), but they were lower than the fruit weights of black mulberry fruits grown in Turkey (Koyuncu *et al.*, 2004; Gunes and Cekic, 2004; Ercisli and Orhan, 2008) and black mulberry variety in Turpan of China (Li *et al.*, 2020). In addition, ‘Zheza 2’ exhibited the highest FL ( $26.20 \pm 0.77$  mm) and FD ( $15.53 \pm 0.24$  mm). In contrast, ‘Tang 10×Lun 109’ exhibited the lowest FL ( $21.83 \pm 0.96$  mm) and FD ( $13.60 \pm 0.46$  mm). Variations in fruit

lengths and diameters have also been reported in black mulberry in different region by literature above. However, in this work, the lower values have been noted. Considerable variations of fruit weights and sizes of mulberry are usually related to the genotypes and growing environments, as well as maturity of fruit, for instance, the fruit weights of ‘Mavromournia’ variety largely increased from immature (4 g) to fully ripe fruit (7 g) (Gerasopoulos and Stavroulakis, 1997). This study showed that different rootstocks could obviously affect fruit weight and size of mulberry fruits. Moreover, there were no significant differences among the 8 rootstocks in terms of fruit shape indexes of ‘Zijing’ mulberry.

**Table 1.** Fruit physical parameters of ‘Zijing’ mulberry on different rootstocks

Rootstock	FW (g)	FL (mm)	FD (mm)	FSI
‘Guisang 5’	2.74 ± 0.15 b	24.50 ± 0.92 ab	14.53 ± 0.53 ab	1.69 ± 0.03 a
‘Guisang 6’	2.58 ± 0.06 b	23.95 ± 1.80 abc	14.10 ± 0.92 b	1.70 ± 0.03 a
‘Guisangyou 12’	2.68 ± 0.10 b	23.16 ± 1.38 bc	14.34 ± 0.23 ab	1.61 ± 0.07 a
‘Tang 10 × Lun 109’	2.60 ± 0.10 b	21.83 ± 0.96 c	13.60 ± 0.46 b	1.61 ± 0.03 a
‘Yuesang 11’	2.53 ± 0.08 b	22.97 ± 0.81 bc	14.16 ± 0.69 b	1.62 ± 0.04 a
‘Yuesang 51’	2.73 ± 0.12 b	23.59 ± 0.65 bc	14.39 ± 0.42 ab	1.64 ± 0.02 a
‘Zheza 2’	3.06 ± 0.19 a	26.20 ± 0.77 a	15.53 ± 0.24 a	1.69 ± 0.03 a
Zhenzhubai seedlings	2.70 ± 0.02 b	23.96 ± 0.30 abc	14.41 ± 0.37 ab	1.66 ± 0.04 a

The different letter(s) in each column indicate significant differences among means within each rootstock at  $p < 0.05$  (Duncan test). FW (fruit weight), FL (fruit length), FD (fruit diameter), FSI (fruit shape index).

#### *Biochemical parameters of mulberry fruit*

TSS (containing sugars, salts, protein, acids, etc.) is a fundamental index for assessing fruit quality (Oz and Ulukanli, 2014). In this study, the rootstocks significantly affected TSS of mulberry fruits (Table 2). TSS of 8 rootstocks of mulberry ranged from 10.96 to 13.93 °Brix. Both of ‘Guisangyou 12’ and ‘Zheza 2’ exhibited the highest and similar TSS of 13.93 °Brix and 13.44 °Brix, respectively, whereas ‘Guisang 5’ had the lowest and did differ significantly from both above rootstocks. Generally, the refractometer, which was chosen for measuring TSS, has been widely used as a rapid method to determine sugar content of preharvest fruit. However, Hale *et al.* (2005) reported that TSS was poorly correlated with sucrose and total sugar in sweet corn. Therefore, we further analyzed the content of sugar and acid.

The sensory quality of fruit mainly depends on the contents of sugars and organic acids (Colaric *et al.*, 2005). Generally, the higher the sugar content, the sweeter the fruit. In this work, we observed significant differences of SSC, RSC and TA among the 8 rootstocks (Table 2). ‘Yuesang 51’ had the highest SSC (9.30%), RSC (8.08%) and the lower TA (1.21%). Conversely, the lower level of SSC (7.74%), RSC (6.83%) and the highest TA (1.58%) were observed in ‘Zheza 2’. Moreover, ‘Guisang 5’ exhibited the minimum value of SSC (7.45%) and RSC (5.15%), and the lower TA (1.40%) as compared to the fruits in ‘Zheza 2’. It could be inferred that the SSC was positively correlated with RSC and negatively correlated with TA in mulberry fruit. These results coincided with findings in Zhao *et al.* (2019), which reported that SSC accumulation and rapid decrease of TA were mainly in the third stage (rapid growth stage II) and reached the peak and bottom, respectively, at full maturity.

The ratio of soluble sugars to organic acids, two of the major metabolites in fleshy fruits, has an important impact on balancing the sweetness and tartness of fruit. But it can only be regarded as a subjective parameter to evaluate the consumer’s perception of fruit sweetness rather than a horticultural trait, on account of a tight link between sugar and acid metabolism (Qiao *et al.*, 2017). As shown in Table 2, obvious differences in SSC/TA were discovered among the mulberry rootstocks. The highest level of SSC/TA was detected in ‘Yuesang 51’, while ‘Guisang 5’ and ‘Zheza 2’ exhibited the lowest and similar values of SSC/TA.



**Table 2.** Fruit biochemical parameters of ‘Zijing’ mulberry on different rootstocks

Rootstock	TSS (°Brix)	SSC (%)	RSC (%)	TA (%)	SSC/TA
‘Guisang 5’	10.96 ± 0.29 d	7.45 ± 0.25 c	5.15 ± 0.37 e	1.40 ± 0.02 b	5.31 ± 0.12 d
‘Guisang 6’	11.69 ± 1.04 cd	7.79 ± 0.14 bc	6.63 ± 0.08 d	1.23 ± 0.05 cd	6.34 ± 0.36 bc
‘Guisangyou 12’	13.93 ± 0.45 a	8.12 ± 0.28 b	7.31 ± 0.06 c	1.32 ± 0.06 bc	6.16 ± 0.11 c
‘Tang 10 × Lun 109’	12.11 ± 0.26 c	8.13 ± 0.23 b	7.52 ± 0.17 bc	1.18 ± 0.04 d	6.88 ± 0.24 b
‘Yuesang 11’	12.31 ± 0.11 c	8.29 ± 0.37 b	7.74 ± 0.03 ab	1.19 ± 0.06 d	6.95 ± 0.33 b
‘Yuesang 51’	12.67 ± 0.42 bc	9.30 ± 0.36 a	8.08 ± 0.12 a	1.21 ± 0.04 d	7.68 ± 0.25 a
‘Zheza 2’	13.44 ± 0.48 ab	7.74 ± 0.28 bc	6.83 ± 0.17 d	1.58 ± 0.01 a	4.90 ± 0.20 d
Zhenzhubai seedlings	12.16 ± 0.41 c	8.05 ± 0.07 b	6.92 ± 0.13 d	1.16 ± 0.07 d	6.93 ± 0.50 b

The different letter(s) in each column indicate significant differences among means within each rootstock at  $p < 0.05$  (Duncan test). TSS (total soluble solid content), TA (titratable acidity), SSC (soluble sugar content), RSC (reducing sugar content).

#### *Soluble protein content and antioxidant activity of mulberry fruit extracts*

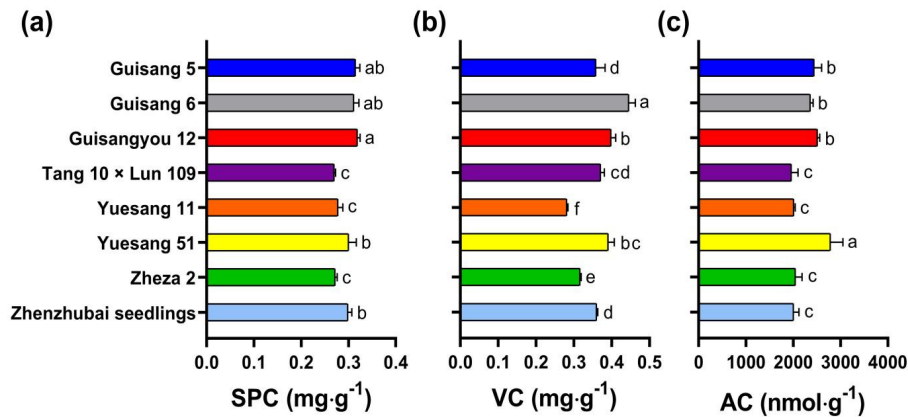
As shown in Figure 2, significant difference was found in SPC, VC and AC among the different rootstocks. ‘Guisangyou 12’, ‘Guisang 5’ and ‘Guisang 6’ showed the highest and similar levels of SPC (0.32 mg·g<sup>-1</sup>, 0.31 mg·g<sup>-1</sup>, 0.31 mg·g<sup>-1</sup>, respectively), whereas ‘Tang 10 × Lun 109’, ‘Zheza 2’ and ‘Yuesang 11’ exhibited the lowest and statistically similar values of SPC (Figure 2a). In addition, the vitamin C content of the fruit varied from 0.28 to 0.45 mg·g<sup>-1</sup> (Figure 2b). The highest VC (0.45 mg·g<sup>-1</sup>) was detected in ‘Guisang 6’, which is significantly different from other rootstocks, and ‘Yuesang 11’ showed the lowest VC. We found that all the rootstocks except ‘Yuesang 11’ and ‘Zheza 2’ had higher Vitamin C content than those detected in Iqbal *et al.* (2010), which reported 25.2-32.25 mg/100 g Vitamin C in four mulberry cultivars. Also, lower levels of vitamin C content were reported in other mulberry studies (Gungor and Sengul, 2008; Ercisli and Orhan, 2008; Ercisli *et al.*, 2010; Eyduran *et al.*, 2015). Notably, vitamin C content of juice sack in citrus ranged from 25.4 to 45.3 mg/100 g FW (Abeyasinghe *et al.*, 2007). Therefore, in addition to fruit of *Actinidia* and *Citrus* species, mulberry fruit also has high vitamin C content and could be considered as excellent sources of vitamin C.

The healthy functions and potential pharmacological properties including anti-oxidative, anti-tumour, hypolipidemic, neuroprotective and anti-inflammatory effects of anthocyanin have been widely recognized and studied (Stintzing and Carle, 2004; Kang *et al.*, 2006; Huang *et al.*, 2011; Sirikanchanarod *et al.*, 2016). Furthermore, the appealing colour of mulberry fruit is mainly attributed to anthocyanin concentration and purple-coloured mulberry fruit usually contains the highest levels of anthocyanin (Aramwit *et al.*, 2010). In the present study, AC ranged from 1960.29 nmol·g<sup>-1</sup> (‘Tang 10 × Lun 109’) to 2785.69 nmol·g<sup>-1</sup> (‘Yuesang 51’) (Figure 2c). Importantly, the AC of Zhenzhubai seedlings, ‘Zheza 2’ and ‘Yuesang 11’ showed similar levels with that of ‘Tang 10 × Lun 109’. Comparison the results with the previous studies is very difficult due to different extracts methods, units usages, measuring instrument, cultivars, maturity stage, growing season, environmental conditions, postharvest storage conditions (Bae and Suh, 2007; Song *et al.*, 2009; Aramwit *et al.*, 2010; Tabakoglu and Karaca, 2018; Guo *et al.*, 2019).

#### *Correlation analysis of fruit quality traits*

The correlation analysis of the fruit quality traits was performed, and the correlation indexes were showed in Table 3. The fruit weight and soluble sugar content are the important indicators for assessment and classification. It can be found that fruit weights had an extremely significant positive relationship with fruit lengths ( $r = 0.848$ ,  $p = 0.008$ ) and titratable acidity ( $r = 0.862$ ,  $p = 0.006$ ), but negatively correlated with SSC/TA ( $r = -0.648$ ,  $p = 0.083$ ), which indicated that the bigger fruit, the higher titratable acidity. Besides, soluble sugar content had a significant positive correlation with reducing sugar content ( $r = 0.817$ ,  $p = 0.013$ ) and SSC/TA ( $r = 0.810$ ,  $p = 0.015$ ), but it has a negative correlation with TA ( $r = -0.482$ ,  $p = 0.227$ ). It could

be inferred that the metabolism of sugars and acids is closely related. The higher the soluble sugar content, the higher the reducing sugar content and the lower the titratable acidity. Recent genetic evidence has revealed a possible mechanistically distinct class of genes that may potentially be involved in maintaining fruit sugar/acid ratios and/or responding to the cellular sugar/acid ratio status, rather than regulating sugar or acid metabolism alone (Qiao *et al.*, 2017).



**Figure 2.** Fruit soluble protein content and antioxidant activity of ‘Zijing’ mulberry fruit extracts on different rootstocks. (a) SPC (soluble protein content) (b) VC (vitamin C content) (c) AC (anthocyanin content)  
The different letter(s) in each column indicate significant differences among means within each rootstock at  $p < 0.05$  (Duncan test)

**Table 3.** Correlation analysis of the fruit quality traits

	FW	FL	FD	FSI	TSS	SSC	RSC	TA	SSC/TA	SPC	VC	AC
FW	1											
FL	0.848**	1										
FD	0.920**	0.943**	1									
FSI	0.450	0.800*	0.555	1								
TSS	0.400	0.114	0.377	-0.397	1							
SSC	-0.193	-0.375	-0.227	-0.516	0.307	1						
RSC	-0.240	-0.480	-0.275	-0.700	0.556	0.817*	1					
TA	0.862**	0.812*	0.852**	0.480	0.294	-0.482	-0.473	1				
SSC/TA	-0.648	-0.707*	-0.661	-0.560	-0.051	0.810*	0.724*	-0.901**	1			
SPC	-0.186	0.050	-0.075	0.275	-0.141	-0.096	-0.420	-0.064	-0.051	1		
VC	-0.230	-0.175	-0.346	0.177	-0.100	0.071	-0.090	-0.252	0.172	0.639	1	
AC	0.005	0.045	0.024	0.078	0.068	0.442	-0.008	0.024	0.156	0.707	0.557	1

\* and \*\* significant at 5 % and 1 % level, respectively. FW (fruit weight), FL (fruit length), FD (fruit diameter), FSI (fruit shape index), TSS (total soluble solid content), TA (titratable acidity), SSC (soluble sugar content), RSC (reducing sugar content), SPC (soluble protein content), VC (vitamin C content), AC (anthocyanin content).

#### Evaluation of results by PCA

It is difficult to select a suitable combination of rootstock-scion according to a single or a few indicators, so it is necessary to comprehensively evaluate fruit quality traits through PCA (Wang and Xing, 2017; Zhao *et al.*, 2020). Based on all the collected data for the fruit quality parameters, it is theoretically possible to consider the standardized values as variables representing fruit quality. Then, according to the eigenvalues of the correlation matrices, PCA was used to obtain the total variance explained by the contribution rate and

cumulative contribution rate interpretation, as well as the component loading matrix (Supplementary Table S2).

**Table 4.** The score and rank of the comprehensive fruit quality parameters among different rootstocks

Rootstock	Q1	Q2	Q3	Q	Rank
'Guisang 5'	-0.565	0.894	0.979	0.117	7
'Guisang 6'	0.179	0.910	1.209	0.499	4
'Guisangyou 12'	0.393	0.179	1.762	0.527	2
'Tang 10 × Lun 109'	0.987	-0.233	0.643	0.507	3
'Yuesang 11'	0.751	-0.427	0.722	0.370	5
'Yuesang 51'	0.819	0.074	2.035	0.744	1
'Zheza 2'	-1.191	-0.641	1.220	-0.472	8
Zhenzhubai seedlings	0.313	0.153	0.973	0.347	6

In this analysis, three components were extracted based on the criterion that the eigenvalues are larger than one. The accumulative contribution rate of first three principal components was 85.49 %. Particularly, PC1 explained 45.34 % of the total variance. Moreover, PC1 was found to be associated with the fruit size indicators (fruit weight, fruit length, fruit diameter and fruit shape index) and the fruit flavour index (soluble sugar content, reducing sugar content, titratable acidity and the ratio of soluble sugar content/ titratable acidity), PC2 was found to be soluble protein content and Vitamin C content, and PC3 was found to be anthocyanin content. These results were consistent with previous study (Zhao *et al.*, 2020). As shown in Table 4, 'Yuesang 51' was the first in the ranks of comprehensive fruit quality based on the result of PCA due to the higher score of mulberry's pigment factor, followed by 'Guisangyou 12', and 'Zheza 2' was clearly last.

## Conclusions

The results of this study revealed that rootstock had significant effects on mulberry fruit quality. Although 'Zijing' mulberry grafted on 'Zheza 2' had the highest fruit weight and size, it showed the higher titratable acidity and the lower levels of other compounds than other rootstocks. Therefore, 'Zheza 2' is not recommended as an ideal rootstock for mulberry cultivar. In contrast, 'Yuesang 51' could be considered as a suitable rootstock for 'Zijing' mulberry due to the highest sugar content (soluble sugar and reducing sugar), anthocyanin content and the lowest titratable acidity. Furthermore, the results of PCA showed that the quality of 'Yuesang 51' ranked first, followed by 'Guisangyou 12'. This study provided significant progress for effects of rootstocks on mulberry fruit quality and selectable rootstocks in mulberry breeding projects.

## Authors' Contributions

Conceptualization, RLM and NZ; methodology, RLM, NZ and ZXD; software, RLM and NZ; validation, RLM and NZ; formal analysis, RLM and NZ; investigation, RLM, NZ and YZ; resources, RLM, NZ, YZ, YL and CZ; data curation, RLM and NZ; writing original draft preparation, RLM and NZ; writing review and editing, RLM, NZ, ZXD, ZXZ, YL, CZ and CY; visualization, RLM and NZ; project administration, QJ; funding acquisition, CY; All authors read and approved the final manuscript.



### **Ethical approval** (for researches involving animals or humans)

Not applicable.

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### **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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