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Characteristics of *Schisandra chinensis* (Turcz.) Baillon Collected in Habitats of South Korea

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Abstract: *Schisandra chinensis* (Turcz.) Baill. has been traditionally used as a medicine for bronchial tube ailments in Northeast Asia. Recently, the demand for *S. chinensis* has increased for use as food and medicine because of the lignans found in its fruits. However, the species germplasm collection has rarely been studied to evaluate their phenotypic traits in Korea. In this study, 96 accessions of *S. chinensis* were collected in South Korea for two years to evaluate their phenotypes, and principal component analysis (PCA) and cluster analysis were conducted. Significant variations among the measured phenotypic traits were observed. The total bunch weight produced from a single tree ranged from 109 to 5844 g; the contents in schizandrin, gomisin A, and gomisin N—three types of lignan—were 2.2–14.5, 0.9–9.8, and 2.1–12.2 mg/g, respectively. Gomisin N positively correlated with flowering traits (flowering start, period, and peduncle length) and leaf characteristics (leaf width and length). PCA and cluster analyses revealed four clusters among the 96 *S. chinensis* accessions. These results provide valuable information for systematic characterization of species germplasm collections and tools for further research.

Keywords: *Schisandra chinensis*; schizandrin; gomisin A; gomisin N; principal component analysis; cluster analysis; phenotypic traits

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1. Introduction

Schisandra species belong to the Schisandraceae family, which includes 23 species broadly distributed worldwide. For example, *S. glabra* is indigenous to North America, while all the other species are in East Asia [1]. Two species are found in Korea: *S. chinensis* (Tucrz.) Baillon is distributed in the Korean peninsula, whereas *S. repanda* (Siebold and Zucc.) Radlk is only found on Jeju Island [2]. *S. chinensis* is a woody perennial plant with bunch fruits and hermaphrodite, entomophilous, and monosexual flowers. The origin of the species name *Schisandra* combines the Greek words "schizein" and "andros", meaning splitting and man, respectively, which refers to the separate male and female flowers as the species is dioecious. A study reported that gender variation for *S. chinensis* remained unclear [3]. In Korea, *S. chinensis* is called Omija, which refers to the plant's five flavors: sour, sweet, spicy, bitter, and salty. Omija is a representative medicinal crop with 965 ha cultivated in Mungyeong, Gyeongsangbuk-do Province, out of 1891 ha in South Korea [4].

S. chinensis has been used as an herbal ingredient since ancient times, and its main application is in healing physical organs, controlling neuropsychiatric symptoms, and regulating non-physiologic actions in the body [5]. The fruit is used as a tonic, astringent, and cough medicine. In addition, its seeds, roots, and stems are used to stimulate the circulation and respiratory systems [6]. The health benefits and medicinal effects of *Schisandra* spp. include hepatoprotective activity against damage caused by long-term acetaminophen use [7,8], preventing HIV-1 and TMV infections [9,10] and cancer [11–14], protection against neuronal cell death and cognitive impairment [15], antidepressant qualities, improvement of learning abilities [16] and nerve stability, and fighting insomnia by modifying the serotonin system [17]. Lignan, functional components of the fruit extract of *Schisandra* spp., inhibit the differentiation of adipocytes and fat cells in mice [18–20]. The latest research on lignans focused on their role in skin protection and diet. Lignans have excellent stability and antioxidant effects to protect the skin from UV rays and exhibit anti-inflammatory effects [21,22].

Accessions of *S. chinensis* were explored in the M. Grishko National Botanical Garden (Ukrainian NAS, Kyiv) collection, and the cultivar "Sadovy 1" was developed [23]. Kozo, [24] reported that *S. chinensis* was the only Schisandraceae species pollinated by insects. Liang et al. [25] indicated that *S. henryi*, distributed in southern central China, was pollinated with the help of *Aphaenogaster megommata* (ants) for further ovarian maturation. Zhao et al. [26] reported that shade intensity was negatively correlated with the female ratio of *S. chinensis*. In the cultivated population, plant age was positively correlated with flower production and female ratio.

A previous study selected 16 promising accessions in Chungju, South Korea, by examining the plants' characteristics [27]. Fruit characteristics, total flavonoid and polyphenol contents, and major components of *Schisandra* accessions were reported [28]. Characteristics of the flowering period, tissue culture, and dormancy-breaking methods were reported for *S. repanda* [29]. Only one variety of *S. chinensis*, named "Chungsun", was developed by selecting the collected accessions in South Korea [30]. Most farmers have been using open-pollinated flowers to produce seedlings for cultivation. Cross-pollination of *S. chinensis* causes variations in fruit production and quality, including lignan contents. Therefore, selection, germplasm evaluation, and combining ability of selected genotypes in breeding programs of *S. chinensis* are required to increase the productivity and lignan content of *S. chinensis* plants.

In this study, 96 *Schisandra* accessions were collected from seven regions in South Korea and planted. Their growth, flowering characteristics, fruit traits including main lignan components, and the presence of pathogens were evaluated and the relationships among these parameters were assessed. This study provides information and tools for the selection and breeding of high lignan-containing cultivars.

2. Materials and Methods

Ninety-six *S. chinensis* accessions were collected from Bonghwa in Gyeongsangbuk-do (n = 20), Mungyeongsi in Gyeongsangbuk-do (n = 23), and Yeongyang in Gyeongsangbuk-do (n = 19), Danyang in Choungchungbuk-do (n = 12), Yeongwol in Gangwon-do (n = 10), Taebaek in Gangwon-do (n = 10), and Jinan in Jeollabuk-do (n = 2) in South Korea between 2014 and 2015. The information on the number of accessions and collecting locations and altitudes is displayed in Table 1.

Table 1. Number of accessions, collection site and altitude of *Schisandra chinensis* accessions collected from habitat in South Korea.

Number of Accessions	Altitude (m)	Collection Site
8	361	Bongseongmyeon, Bonghwagun, Gyeongsangbukdo
5	450	Jaesanmyeon, Bonghwagun, Gyeongsangbukdo
4	433	Beopjeonmyeon, Bonghwagun, Gyeongsangbukdo
3	419	Chunyangmyeon, Bonghwagun, Gyeongsangbukdo
23	520	Dongnomyeon, Mungyeongsi, Gyeongsangbukdo
12	687	Daegangmyeon, Danyanggun, Chungcheongbukdo
10	706	Sangdongeup, Yeongwolgun, Gangwondo
10	878	Hwangjidong, Taebaeksi, Gangwondo
8	359	Irwolmyeon, Yeongyanggun, Gyeongsangbukdo
11	545	Subimyeon, Yeongyanggun, Gyeongsangbukdo
2	471	Jinaneup, Jinangun, Jeollabuk-do

2.1. Design of Field Trial

To cultivate *S. chinensis* accessions, an evaluation field with a trapezoidal post (2 m wide and 2 m high; Figure 1A) was built at the Bonghwa Medicinal Crops Research Center (longitude 128°48′36″, latitude 36°53′54″) in 2014. Triplicates of the collected accessions were planted on 9 March 2015. Before planting, three suckers with a length of 20 cm and a diameter of 0.5 cm were cut from each accession (Figure 2). The first block was planted sequentially, while the second and third blocks were planted randomly. Single plants from each plot were grown for the evaluation of phenotypic measurements. The planting was done in rows of 2 m, and accessions were separated by 2.5 m with one plant per hill (Figure 1). Agronomic traits were assessed from 2018 to 2019.

Figure 1. *Schisandra* life cycle (**A**); trapezoidal post installation, (**B**); sucker planting, (**C**); first year growth after sucker planting, (**D**); second year growth after sucker planting, (**E**); third year growth after sucker planting, (**F**); trapezoidal post cultivation of *Schisandra chinensis*.

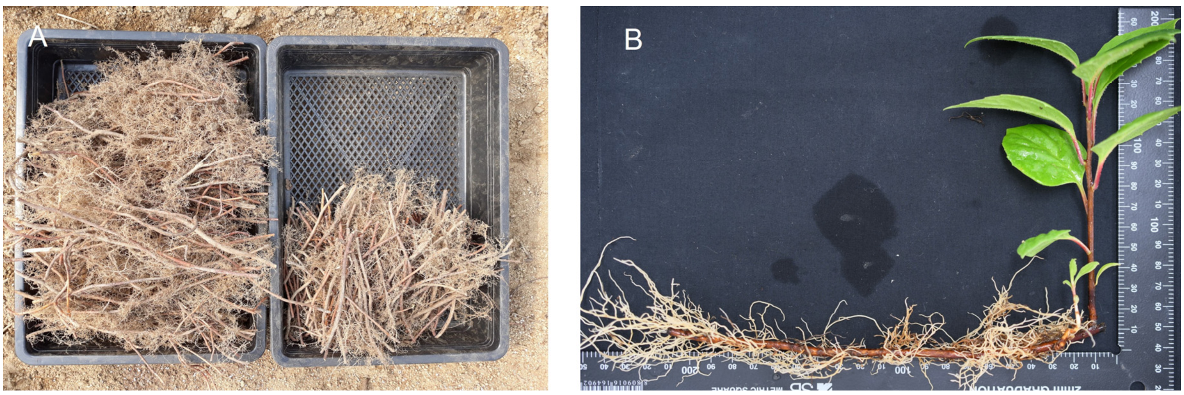

Figure 2. Sucker propagation of *Schisandra chinensis.* (**A**): cutting of sucker, (**B**): shooting of sucker.

2.2. Evaluation of Agronomic Traits

Seventeen phenotypic traits of the 96 accessions were observed for two years between 2018 and2019. The peduncle length (PL) was gauged using a Vernier caliper (500-182-30, Mitutoyo, Kawasaki, Japan). The flowering start (FS) was calculated from 1 January to the day of 10% flowering, and the flowering period (FP) was calculated from 1 January to the day of 50% flowering. The frequency of female flowers (FFF) was measured by examining the proportion of female and male flowers of a tree during flowering. The leaf width (LW) and length (LL) were investigated using the largest leaves of the longest shoot during flowering. Fruit maturity (FM) was measured when 70–80% of the entire tree matured and discolored. For powdery mildew (PM) caused by *Microsphaera* sp. and anthracnose (ANT) by *Colletotrichum gloeosporioides*, the ratio of infected leaf area was measured by visual rating at the peak blooming period in mid-June. The visual rating of infection was calculated by dividing the infected leaf area by the total leaf area and multiplying the result by 100. The fruit length (FL) and width (FW) were measured on the widest side of the fruit bunch from twenty fruit samples of each accession. The fruit number (FN) was defined by counting the fruits on each bunch. The bunch weight (BW) was calculated simply by weighing each bunch, and the total bunch weight (TBW) corresponds to the weight of the total number of bunches per plant. After examining the coloration degree of fully bloomed flowers, flowers were classified as white, light pink, or dark pink. Photographs of flowers and pistils were taken using an imaging device (Leica DFC 500, Leica, Wetzlar, Germany). Pictures of pollen were magnified 50 times with a dissection microscope (Stemi 2000-C, Zeiss, Jena, Germany). Forty pollen grains were measured in this study.

2.3. Evaluation of Schizandrin, Gomisin A, and Gomisin N

For the analysis of schizandrin, gomisin A, and gomisin N, three lignans found in *S. chinensis*, approximately 100 g of fruits harvested from each plot were first stored in a cryogenic refrigerator (Thermo Fisher Scientific, Waltham, MA, USA), and frozen fruits were ground with a grinder and dried with a freeze dryer (Labconco, Kansas City, MO, USA). The dried samples were pulverized with a mortar again to even the particles, and 0.5 g of each pulverized sample was transferred to an Erlenmeyer flask with 25 mL of methanol and processed through an Ultrasonic Cleaner (Crest Ultrasonics Corporation, Ewing Township, NJ, USA) equipment. Next, the samples were heated to 30 °C by ultrasonic waves and extracted twice for 30 min. After filtration using a 25 mm/0.20 µL syringe filter (Chromdisc), samples were analyzed by HPLC (PerkinElmer, Waltham, MA, USA) using a Brownlee-validated C18 column (250.0 mm × 4.6 mm). The standard chemicals for schizandrin ($C_{24}H_{32}O_7$), gomisin A ($C_{23}H_{28}O_7$), and gomisin N ($C_{23}H_{28}O_6$) were purchased from Wako Pure Chemical. The mobile phase was composed of water and CH_3CN (30:70, *v*/*v*), and the flow rate was fixed at 1.0 mL per minute. The UV detector was set at 254 nm, and the analysis time per sample was 30 min, so no interference occurred between the samples. All accessions were analyzed in triplicate.

2.4. Statistical Analysis

Statistical analysis was performed with SAS (version 9.4 and Enterprise Guide 7.1, Statistical Analysis System, 2013, Cray, NC, USA). When the analysis of variance (ANOVA) indicated significance, the least significant difference (LSD) test was applied to compare means with $p < 0.05$. Significant differences between agriculture traits were analyzed with Pearson's correlation coefficient, and the significance level was set at 5% ($p < 0.05$).

2.5. PCA

Thirteen traits obtained over two years were used for principal component analysis (PCA). The PCA was performed using the PRINCOMP procedure of SAS, and the results are presented as a scatter diagram generated by SAS.

2.6. Hierarchical Cluster Analysis

All investigations were conducted based on the PCA of the 96 *S. chisandra* accessions. Cluster analysis was performed with a hierarchical bunch analysis, with the observed values classified into several clusters according to their degree of similarity. The appropriate number of clusters was determined through optimal separation cluster analysis using R-Square (RSQ) and RSQ/(1-RSQ) measures. The R-Square and RSQ/(1-RSQ) values indicate that each variable is a bunch. They were used as indicators to judge the degree of influence on the formation of clusters [31]. Cluster analysis was performed using the SAS FASTCLUS procedure. The results of the bunch analysis were generated by CANDISC and GPLOT of SAS.

3. Results

3.1. ANOVA for Phenotypic Measurements

This study aimed to understand the genetic diversity of 96 *S. chinensis* accessions through 17 phenotypic measurements. ANOVA was conducted to identify the effect of genotype and year, and their interactive effect on 17 phenotypic traits for two years between 2018 and2019 (Table 2). ANOVA results indicated a significant effect of years on 10 traits, including start flowering ($p \leq 0.001$), flowering period ($p \leq 0.001$), leaf width ($p \leq 0.001$), leaf length ($p \leq 0.001$), peduncle length ($p \leq 0.001$), fruit length ($p \leq 0.05$), fruit maturity ($p \leq 0.001$), schizandrin ($p \leq 0.001$), gomisin A ($p \leq 0.001$), and gomisin N ($p \leq 0.001$). The genotype had a significant effect on 11 traits, including start flowering ($p \leq 0.001$), flowering period ($p \leq 0.01$), frequency of female flower ($p \leq 0.01$), leaf width ($p \leq 0.001$), leaf length ($p \leq 0.001$), fruit length ($p \leq 0.05$), fruit width ($p \leq 0.001$), fruit number ($p \leq 0.001$), fruit maturity ($p \leq 0.001$), schizandrin ($p \leq 0.001$), gomisin A ($p \leq 0.001$), gomisin N ($p \leq 0.001$). In addition, the interaction of genotype and year significantly affected schizandrin, gomisin A, and gomisin N contents ($p \leq 0.001$).

Table 2. Analysis of variance for agronomic traits of 96 *Schisandra chinensis* accessions across 2 years.

Source of Variation	DF	F Value				
		FS [(1)]	**FP**	**FFF**	**LW**	**LL**
Year (Y)	1	775.92 ***	832.54 ***	1.36 ns [(2)]	97.70 ***	160.94 ***
Genotype (G)	95	1.68 ***	4.88 **	2.17 **	9.46 ***	4.55 ***
Y × G	95	0.28 ns	0.06 ns	0.04 ns	0.72 ns	1.32 ns
Source of Variation	**DF**	**F Value**				
		PL	**PM**	**ANT**	**FL**	**FW**
Y	1	74.64 ***	0.79 ns	2.59 ns	5.15 *	0.25 ns
G	95	1.40 ns	1.42 ns	0.81 ns	1.81 *	2.64 ***
Y × G	95	0.72 ns	1.12 ns	0.00 ns	0.88 ns	0.50 ns
Source of Variation	**DF**	**F Value**				
		FN	**BW**	**TWB**	**FM**	
Y	1	0.66 ns	0.43 ns	1.93 ns	31.12 ***	
G	95	1.22 ***	12.68 ns	1.29 ns	25.30 ***	
Y × G	95	0.50 ns	0.79 ns	0.83 ns	0.15 ns	
Source of Variation	**DF**	**F Value**				
		Schizandrin		**Gomisin A**		**Gomisin N**
Y	1	718,521.00 ***		405,053.00 ***		190,368.00 ***
G	95	975.97 ***		372.59 ***		301.89 ***
Y × G	95	872.38 ***		339.36 ***		268.26 ***
Corrected Total	155					

(1) FS; flowering start (days to flowering was calculated from 1 January to the day 10% of flowering), FFF; frequency of female flower, FP; flowering period (days to 50% flowering was calculated from 1 January to the day 50% of flowering), LW; leaf width, LL; leaf length, PL; peduncle length, PM; powdery mildew, ANT; anthracnose, FL; fruit length, FW; fruit width, FN; fruit number, BW; bunch weight, TWB; total weight of bunch produced from a single tree, FM; fruit maturity(cumulative days after 1 January), (2) ns: not significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3.2. Phenotypic Traits and Lignan Compounds of Schisandra chinensis

The phenotypic traits and lignan compounds of the 96 accessions were investigated from 2018 to 2019, as described above. The flowering start ranged between 122.0 and

128.0 days (average 123.7 days). The flowering period ranged from 126.5 to 131.5 days (average 127.7 days). The male and female flower ratio ranged between 0.0% and 95.0% (average 75.7%). The leaf width and length ranged between 3.9 and 6.9 cm (average 5.4 cm) and 6.4–17.1 cm (average 9.2 cm), respectively. The peduncle length ranged from 1.4 to 4.8 cm (average 2.9 cm). The powdery mildew infection rate was 0.0–25.0% (average 3.5%), while the anthrax infection rate was 0.0–25.0% (average 1.2%). The fruit length and width ranged from 8.0 to 12.8 cm (average 10.2 cm) and 7.8 to 12.3 cm (average of 9.8 cm), respectively. The number of fruits ranged from 14.7 to 36.2 (average of 25.1). The bunch weight ranged from 6.1 to 26.9 g (average 14.2 g), while the total bunch weight ranged from 109.0 to 5844.0 g (average of 1367.5 g). The fruit maturity ranged from 248.5 to 273.0 days (average 260.0 days). *Schisandra* fruits were classified into small (<10 g, Figure 3D), medium (10–20 g, Figure 3E), and large fruits (>20 g, Figure 3F) based on their size.

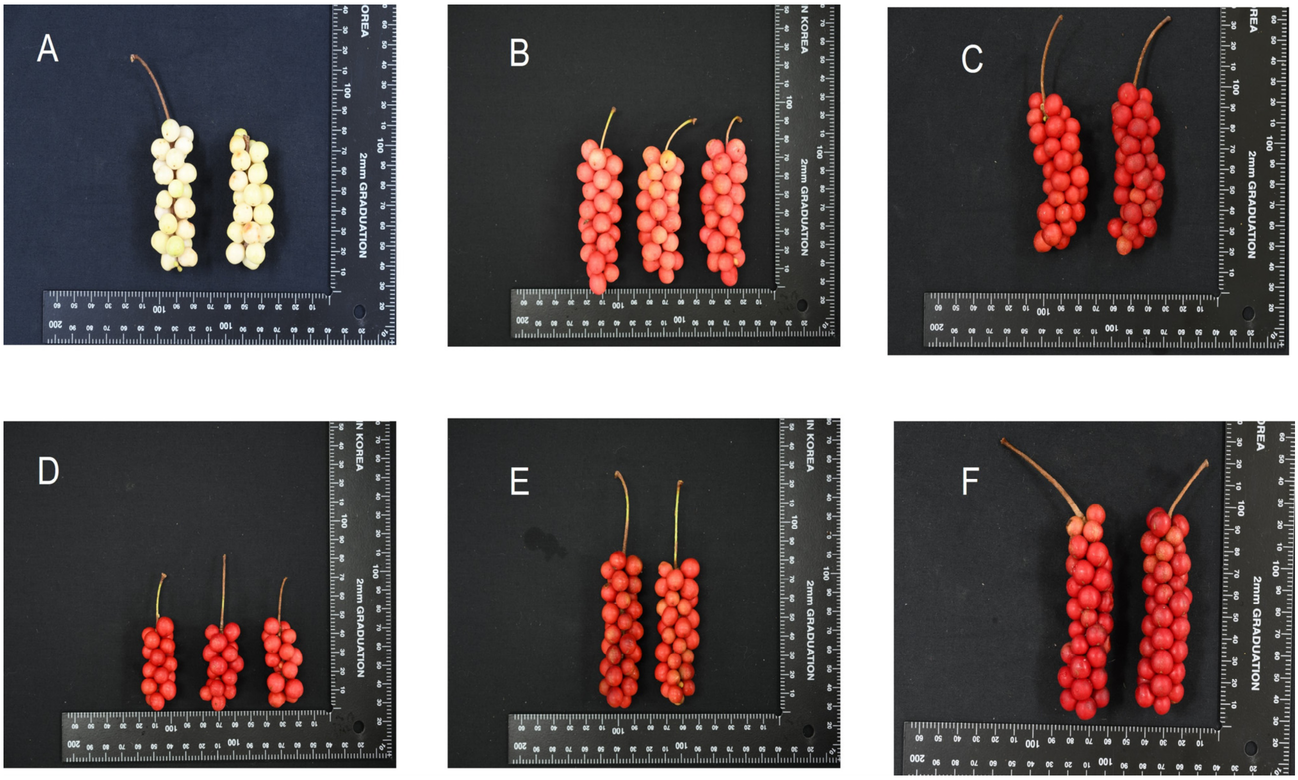

Figure 3. Fruit traits of *Schisandra chinensis* bunch and berry among 96 *Schisandra chinensis* accessions. (**A**); bunch of white fruit (n = 1), (**B**); bunch of pink fruit (n = 7), (**C**); bunch of red fruit (n = 88), (**D**); small (n = 10), bunch, (**E**); middle bunch (n = 81), (**F**); big bunch (n = 5).

Regarding the lignan compounds in the *Schisandra* fruits, schizandrin ranged from 2.2 to 14.5 mg/g (average 6.9 mg/g), gomisin A ranged from 0.9 to 9.8 mg/g (average of 3.2 mg/g), and gomisin N ranged from 2.1 to 12.2 mg/g (average of 5.2 mg/g) (Table 3).

S. chinensis plants have male and female flowers blooming in one tree that can be classified into three colors: dark pink, light pink, and white. Based on the observations, 12 accessions displayed white flowers, 61 had light pink flowers, and 23 displayed dark pink flowers. White flowers are completely distinguished from light pink and dark pink ones (Figure 4; Table 4). Because the stigma of the female flowers has a sticky substance, pollen grains were moved to the stigma and germinated to form pollen tubes. When the pollen tube lengthened, pollen grains were about 20–100 µm and displayed a round shape (Figure 5).

Table 3. Agronomic traits among 96 accessions of *Schisandra chinensis* for 2 years.

Traits (Unit) [(1)]	Maximum	Minimum	Mean ± S.D	C.V. (%)
FS (days)	128.0	122.0	123.7 ± 7.6	1.9
FP (days)	131.5	126.5	127.7 ± 0.4	1.6
FFF (%)	95.0	0.0	75.7 ± 1.8	23.7
LW (cm)	6.9	3.9	5.4 ± 0.0	7.7
LL (cm)	17.1	6.4	9.2 ± 0.1	11.8
PL (cm)	4.8	1.4	2.9 ± 0.5	17.5
PM (%)	25.0	0.0	3.5 ± 0.3	101.6
ANT (%)	25.0	0.0	1.2 ± 0.3	381.3
FL (cm)	12.8	8.0	10.2 ± 0.1	6.1
FW (cm)	12.3	7.8	9.8 ± 0.1	6.8
FN (ea)	36.2	14.7	25.1 ± 0.3	16.9
BW (g)	26.9	6.1	14.2 ± 0.3	22.4
TWB (g/plant)	5844.0	109.0	1367.5 ± 87.5	71.3
FM (days)	273.0	248.5	260.0 ± 2.8	2.0
Schizandrin (mg/g)	14.5	2.2	6.9 ± 0.1	28.3
Gomisin A (mg/g)	9.8	0.9	3.2 ± 0.1	25.9
Gomisin N (mg/g)	12.2	2.1	5.2 ± 0.1	9.5

[(1)] PL; peduncle length, FS; start flowering (days to flowering was calculated from 1 January to the day 10% of flowering), FP; Flowering period (days to 50% flowering was calculated from 1 January to the day 50% of flowering), FFF; frequency of female flower, LW; leaf width, LL; leaf length, PM; powdery mildew, ANT; anthracnose, FL; fruit length, FW; fruit width, FN; fruit number, BW; bunch weight, TWB; total weight of bunch produced from a single tree, FM; fruit maturity (harvest days cumulative after 1 January).

Figure 4. Phenotype of *Schisandra chinense* flower. (**A**–**C**); female flower, (**D**–**F**); male flower. (**A**,**D**); white flower (**B**,**E**); light pink flower, (**C**,**F**); dark pink flower.

Table 4. Distribution of flower color among *Schisandra chinensis* of 96 accessions.

	White	Light Pink	Dark Pink
Accessions	12	61	23

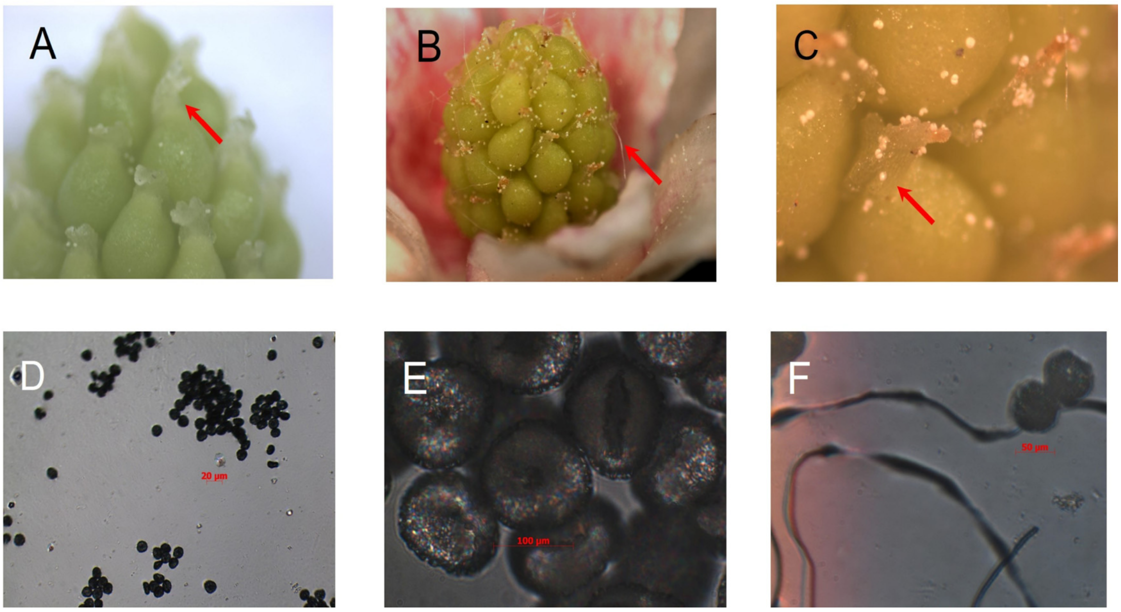

Figure 5. A pistil and pollen of *Schisandra chinense.* (**A**); pistil, (arrowhead) towards the stigma. (**B**); pistil with pollen, (arrowhead) towards pollen tube elongation. (**C**); stigma with pollen, (arrowhead) towards pollen. (**D**,**E**); pollen, (**F**); pollen tube elongation.

3.3. Correlation Analysis of 96 Accessions of Schisandra chinensis

The correlations among 14 phenotypic measurements were investigated (Table 5). The flowering start was significantly correlated with the flowering period ($p \leq 0.001$, $r = 0.990$), frequency of female flowers ($p \leq 0.05$, $r = 0.192$), leaf width ($p \leq 0.001$, $r = 0.516$), leaf length ($p \leq 0.001$, $r = 0.702$), peduncle length ($p \leq 0.001$, $r = -0.518$), bunch weight ($p \leq 0.001$, $r = 0.283$), and fruit maturity ($p \leq 0.01$, $r = 0.243$). The flowering period was significantly correlated with the frequency of female flowers ($p \leq 0.05$, $r = 0.178$), leaf width ($p \leq 0.001$, $r = 0.534$), leaf length ($p \leq 0.001$, $r = 0.708$), peduncle length ($p \leq 0.001$, $r = -0.514$), bunch weight ($p \leq 0.001$, $r = 0.231$), and fruit maturity ($p \leq 0.01$, $r = 0.244$). The frequency of female flowers was significantly correlated with fruit length ($p \leq 0.05$, $r = 0.162$), fruit width ($p \leq 0.01$, $r = -0.213$), fruit number ($p \leq 0.01$, $r = 0.209$), and total bunch weight ($p \leq 0.01$, $r = 0.247$). The leaf width was significantly correlated with the leaf length ($p \leq 0.001$, $r = 0.705$), peduncle length ($p \leq 0.001$, $r = -0.302$), fruit length ($p \leq 0.05$, $r = 0.160$), fruit width ($p \leq 0.05$, $r = 0.160$), and bunch weight ($p \leq 0.001$, $r = 0.283$). The leaf length was significantly correlated with the peduncle length ($p \leq 0.001$, $r = -0.405$) and bunch weight ($p \leq 0.001$, $r = 0.233$). The peduncle length was significantly correlated with the fruit number ($p \leq 0.05$, $r = 0.175$), total bunch weight ($p \leq 0.05$, $r = 0.177$), and fruit maturity ($p \leq 0.01$, $r = -0.212$). The powdery mildew was significantly correlated with the fruit length ($p \leq 0.05$, $r = -0.186$) and bunch weight ($p \leq 0.05$, $r = -0.200$). The anthracnose was significantly correlated with the fruit length ($p \leq 0.05$, $r = -0.186$) and bunch weight ($p \leq 0.05$, $r = -0.200$). The fruit length was significantly correlated with the frequency of female flowers ($p \leq 0.01$, $r = -0.162$), leaf width ($p \leq 0.05$, $r = 0.160$), and powdery mildew ($p \leq 0.05$, $r = -0.186$). The fruit weight was significantly correlated with the bunch weight ($p \leq 0.001$, $r = 0.627$), and the bunch weight was significantly correlated with the total bunch weight ($p \leq 0.001$, $r = 0.321$). Finally, the total bunch weight was significantly correlated with the fruit maturity ($p \leq 0.01$, $r = -0.210$).

Table 5. Correlation for agronomic traits among 96 accessions of *Schisandra chinensis* for 2 years.

Traits	FS [(1)]	FP	FFF	LW	LL	PL	PM	ANT	FL	FW	FN	BW	TW B	FM
FS	1													
FP	0.990 ***	1												
FFF	0.192 *	0.178 *	1											
LW	0.516 ***	0.534 ***	−0.074	1										
LL	0.702 ***	0.708 ***	−0.030	0.705 ***	1									
PL	−0.518 ***	−0.514 ***	−0.013	−0.302 ***	−0.405 ***	1								
PM	−0.121	−0.112	−0.028	−0.107	−0.118	−0.131	1							
ANT	0.111	0.111	0.097	−0.064	0.104	−0.107	0.017	1						
FL	0.110	0.120	−0.162 *	0.160 *	0.069	−0.057	−0.186 *	0.005	1					
FW	0.011	0.025	−0.213 **	0.160 *	0.044	−0.057	−0.122	−0.088	0.812 ***	1				
FN	−0.029	−0.010	0.209 **	−0.002	−0.052	0.175 *	−0.051	−0.002	−0.109	−0.017	1			
BW	0.209 **	0.231 **	−0.023	0.283 ***	0.233 **	−0.014	−0.200 *	−0.020	0.516 ***	0.627 ***	0.630 ***	1		
TWB	−0.140	−0.129	0.247 **	0.079	−0.089	0.177 *	−0.039	0.022	0.007	0.073	0.397 ***	0.321 ***	1	
FM	0.243 **	0.244 **	−0.122	−0.022	−0.031	−0.212 **	−0.075	−0.08	0.134	−0.023	0.083	0.074	−0.210 **	1

[(1)] FS; flowering start (days to flowering was calculated from 1 January to the day 10% of flowering), FP; flowering period(days to 50% flowering was calculated from 1 January to the day 50% of flowering), FFF; frequency of female flower, LW; leaf width, LL; leaf length, PL; peduncle length, PM; powdery mildew, ANT; anthracnose, FL; fruit length, FW; fruit width, FN; fruits number, BW; bunch weight, TWB; total weight of bunch produced from a single tree, FM; fruit maturity(cumulative days after 1 January), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 6 presents the correlation results between the three major lignan compounds and phenotypic traits of *S. chinensis* across two years. Schizandrin content was significantly correlated with the frequency of female flowers ($p \leq 0.05$, $r = 0.223$), fruit maturity ($p \leq 0.01$, $r = 0.233$), and gomisin N ($p \leq 0.01$, $r = 0.234$). Gomisin A was significantly correlated with the flowering start ($p \leq 0.0001$, $r = -0.522$), flowering period ($p \leq 0.0001$, $r = -0.535$), leaf width ($p \leq 0.0001$, $r = -0.346$), leaf length ($p \leq 0.0001$, $r = -0.436$), peduncle length ($p \leq 0.0001$, $r = 0.400$), fruit length ($p \leq 0.01$, $r = -0.256$), fruit width ($p \leq 0.001$, $r = -0.272$), total bunch weight ($p \leq 0.001$, $r = -0.263$), and gomisin N ($p \leq 0.001$, $r = -0.271$). Gomisin N was significantly correlated with flowering start ($p \leq 0.0001$, $r = 0.425$), flowering period ($p \leq 0.0001$, $r = 0.419$), leaf width ($p \leq 0.01$, $r = 0.206$), leaf length ($p \leq 0.0001$, $r = 0.335$), peduncle length ($p \leq 0.01$, $r = -0.234$), fruit number ($p \leq 0.05$, $r = 0.196$), bunch weight ($p \leq 0.05$, $r = 0.245$), schizandrin ($p \leq 0.01$, $r = 0.23$), and gomisin A ($p \leq 0.001$, $r = -0.272$).

Table 6. Correlation between lignan compositions and major traits for 2 years in *Schisandra chinensis*.

Traits [(1)]	Schizandrin	Gomisin A	Gomisin N
FS	0.143	−0.522 ****	0.425 **** [(2)]
FP	0.154	−0.535 ****	0.419 ****
FFF	0.223 *	0.128	0.016
LW	−0.027	−0.346 ****	0.206 **
LL	−0.115	−0.436 ****	0.335 ****
PL	−0.026	0.400 ****	−0.234 **
PM	−0.042	0.102	0.009
ANT	0.151	−0.072	0.102
FL	−0.040	−0.256 **	0.060
FW	−0.112	−0.272 ***	0.077
FN	0.194	0.146	0.196 *
BW	−0.005	−0.263 ***	0.245 *
TWB	0.022	0.083	−0.047
FM	0.233 **	−0.153	0.148
Schizandrin	1	0.006	0.234 **
Gomisin A	0.007	1	−0.272 ***
Gomisin N	0.234 **	−0.271 ***	1

[(1)] FS; flowering start (days to flowering was calculated from 1 January to the day 10% of flowering), FP; flowering period(days to 50% flowering was calculated from 1 January to the day 50% of flowering), FFF; frequency of female flower, LW; leaf width, LL; leaf length, PL; peduncle length, PM; powdery mildew, ANT; anthracnose, FL; fruit length, FW; fruit width, FN; fruit number, BW; bunch weight, TWB; total weight of bunch produced from a single tree, FM; Fruit maturity (cumulative days after 1 January), [(2)] * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

3.4. PCA of 96 Accessions of *Schisandra chinensis*

PCA of 13 traits, including phenotypic traits and lignan compounds, of the 96 *S. chinesis* accessions was performed. Five principal components were determined based on their eigenvalue superior to 1, and the cumulative proportion of the components represented the total variance and was superior to 73% (data not shown). Their eigenvalues and contribution to the total variance are listed in Table 7. The first principal component had an eigenvalue of 2.79 and represented 21.5% of the total variance, the second had an eigenvalue of 2.61 with 20.1% of the total variance, the third had an eigenvalue of 1.73 with 13.3% of the total variance, the fourth had an eigenvalue of 1.46 and accounted for 11.2% of the total variance, and the fifth one had an eigenvalue of 0.941 and accounted for 7.2% of the total variance.

Table 7. Eigenvalues and proportion of principal component to 13 quantitative traits of 96 *Schisandra chinensis* accessions.

Eigenvalues of the Correlation Matrix				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	2.799	0.181	0.215	0.215
2	2.618	0.884	0.201	0.417
3	1.735	0.275	0.133	0.550
4	1.460	0.518	0.112	0.662
5	0.941	0.063	0.072	0.735
6	0.878	0.096	0.068	0.802
7	0.783	0.200	0.060	0.863
8	0.583	0.090	0.045	0.907
9	0.492	0.044	0.038	0.945
10	0.449	0.294	0.035	0.980
11	0.155	0.097	0.012	0.992
12	0.058	0.009	0.005	0.996
13	0.049		0.004	1.000

Table 8 presents the correlations between these five principal components and 13 traits. The first principal component displayed a significant positive correlation with the flowering period, flowering start, gomisin A, bunch weight, schizandrin, fruit maturity, length, and width, leaf length, fruit number, and a negative correlation with the peduncle length, leaf width, and gomisin N. The second principal component presented a significant positive correlation with the fruit number, maturity, width, leaf width, bunch weight, and fruit and peduncle length, and a negative correlation with gomisin N, gomisin A, flowering start, schizandrin, leaf length, and flowering period. The third principal component displayed a significant positive correlation with the peduncle and fruit length, leaf width, gomisin N, bunch weight, leaf length, fruit maturity, flowering period, and flowering start, and a negative correlation with schizandrin, fruit width and number, and gomisin A. The fourth principal component positively correlated with the leaf and peduncle length, flowering start and period, fruit width, length, and number, and a negative correlation with gomisin N, bunch weight, schizandrin, gomisin A, fruit maturity, and leaf width. Finally, the fifth principal component displayed a positive correlation with the leaf length, gomisin A, bunch weight, fruit length and maturity, leaf width, and fruit number, and a negative correlation with the peduncle length, flowering start and period, schizandrin, fruit width, and gomisin N. The correlations are listed in correlation strength order (higher to lower).

The factors that most significantly influenced the formation of the first principal component were the flowering period and start. The peduncle length had the most significant negative influence on the formation of the first principal component. Fruit number and maturity had the most significant influence on the second component, while gomisin N had a negative effect on it. The peduncle length had the most significant positive influence, and schizandrin had the strongest negative effect on the formation of the third principal component. The formation of the fourth principal component was mostly affected by leaf and peduncle length and negatively influenced by gomisin N. Finally, leaf length and gomisin A had the greatest positive influence, and peduncle length had the highest negative effect on the formation of the fifth compound.

Table 8. Five principal components among 13 quantitative traits of 96 *Schisandra chinensis* accessions.

	Eigenvectors				
Traits [1]	**Prin1**	**Prin2**	**Prin3**	**Prin4**	**Prin5**
SF	0.500	−0.121	0.084	0.249	−0.277
FP	0.521	−0.078	0.087	0.227	−0.256
LW	−0.111	0.214	0.358	−0.061	0.047
LL	0.065	−0.091	0.131	0.539	0.617
PL	−0.172	0.063	0.479	0.295	−0.389
FL	0.096	0.135	0.433	0.048	0.089
FW	0.074	0.495	−0.244	0.080	−0.020
FN	0.003	0.558	−0.198	0.019	0.041
BW	0.341	0.152	0.309	−0.338	0.114
FM	0.238	0.511	0.124	−0.104	0.051
Schizandrin	0.331	−0.120	−0.307	−0.227	−0.210
Gomisin A	0.360	−0.152	−0.013	−0.191	−0.503
Gomisin N	−0.073	−0.156	0.345	−0.532	−0.002

[1] SF; start flowering (days to flowering was calculated from 1 January to the day 10% of flowering), FP; flowering period (days to 50% flowering was calculated from 1 January to the day 50% of flowering), LW; leaf width, LL; leaf length, PL; peduncle length, FL; fruit length, FW; fruit width, FN; fruits number, BW; bunch weight, FM; fruit maturity (cumulative days after 1 January).

3.5. Hierarchical Cluster Analysis of 96 Accessions of Schisandra chinensis

Based on the PCA, the four principal components accounted for 66.2% of the total variance. A cluster analysis was performed on 96 *Schisandra* accessions resulting in the four clusters presented in Figure 6. In the cluster analysis, RSQ/(1-RSQ) values were used to classify clusters. Fruit maturity (1.700), peduncle length (1.022), fruit number (0.668), flowering period (0.472), start flowering (0.303), and schizandrin (0.252) affected the formation of clusters in this order (Table 9).

Table 9. Statistical variables for cluster of 13 quantitative traits of 96 *Schisandra chinensis* by cluster analysis.

Traits [1]	**Total STD**	**Within STD**	**R-Square**	**RSQ/(1-RSQ)**
FS	2.327	2.079	0.233	0.303
FP	2.040	1.715	0.320	0.472
LW	0.386	0.387	0.031	0.033
LL	0.686	0.676	0.066	0.071
PL	4.363	3.129	0.505	1.022
FL	0.643	0.648	0.023	0.024
FW	0.642	0.639	0.047	0.050
FN	3.661	2.891	0.400	0.668
BW	3.137	2.808	0.230	0.298
FM	5.497	3.411	0.630	1.700
Schizandrin	1.671	1.523	0.201	0.252
Gomisin A	0.980	0.986	0.028	0.029
Gomisin N	1.745	1.687	0.102	0.113
OVERALL	2.639	2.011	0.441	0.790

[1] FS; start flowering (days to flowering was calculated from 1 January to the day 10% of flowering), FP; flowering period (days to 50% flowering was calculated from 1 January to the day 50% of flowering), LW; leaf width, LL; leaf length, PL; peduncle length, FL; fruit length, FW; fruit width, FN; fruit number, BW; bunch weight, FM; fruit maturity (cumulative days after 1 January).

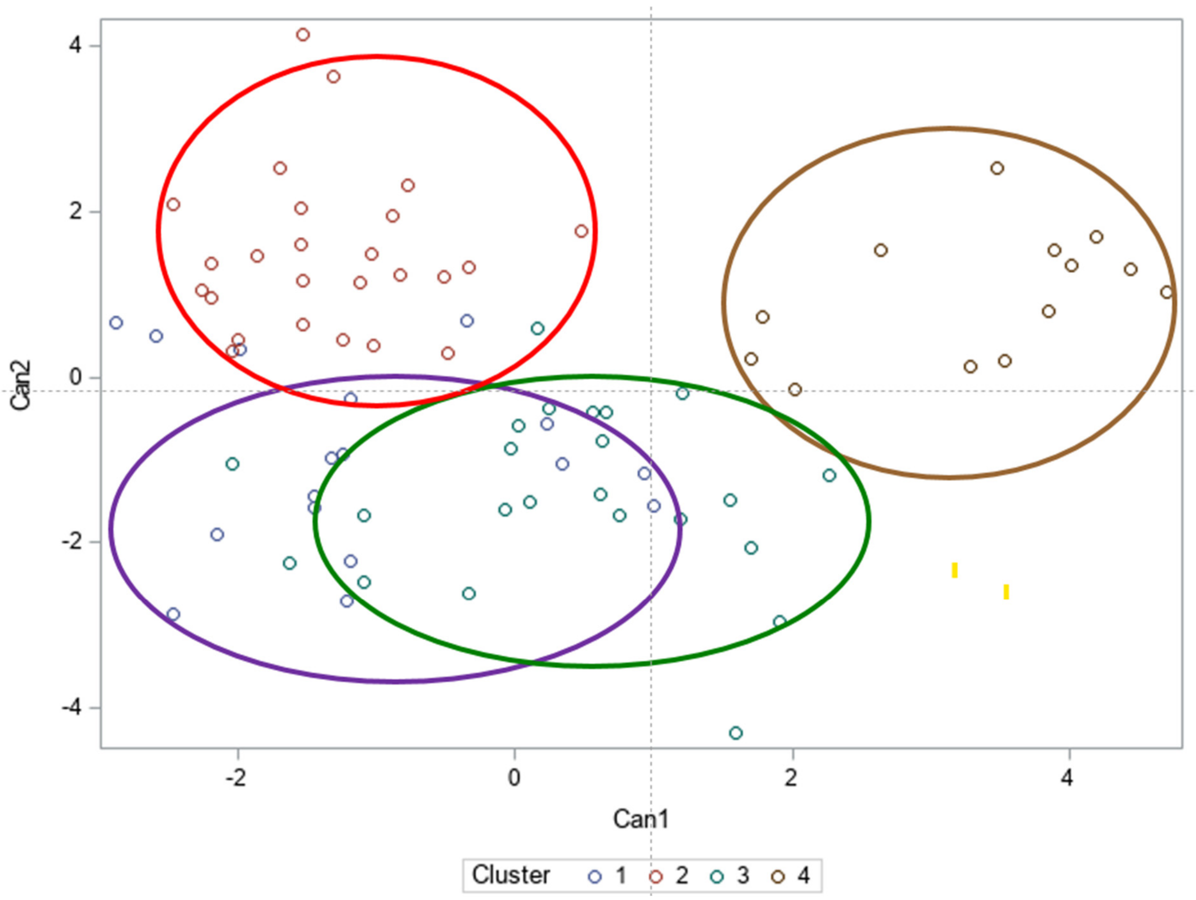

Figure 6. Four clusters based on five principal components of 96 *Schisandra chinensis* accessions by hierarchical clustering method (cluster 1; purple, cluster 2; red, cluster 3; green, cluster 4; brown). Can: canonical variables.

The flowering start was the earliest in cluster 1, with 124 days, and the latest in cluster 4, with 128 days. The flowering period of clusters 1 and 4 was 128 and 132 days, respectively. The flowering start and period in clusters 1 and 3 were significantly earlier than in the other clusters (Figure 7). Although the leaf length was the largest in cluster 2 at 6.0 cm and the lowest in cluster 4 at 4.9 cm, no significant differences were observed between clusters. Leaf length was the highest in cluster 3 at 9.4 cm and the smallest in cluster 1 at 8.4 cm. The peduncle length was the largest in cluster 2, with 39.3 mm. No significant differences were observed in fruit length between clusters. Cluster 1 displayed the highest fruit width at 10.9 mm and cluster 3 the lowest, with 9.5 mm. The fruit number was the highest in cluster 4, with 31.4, and the lowest in cluster 3, with 17.6. The bunch weight was the highest in cluster 4, with 20.9 g, and the lowest in cluster 3, with 8.6 g, which was significantly different from the other clusters. Clusters 1 and 2 displayed an earlier fruit maturation than clusters 3 and 4.

Lignan compounds in the four clusters are displayed in Figure 8. Schizandrin was the highest in cluster 4 with 10.55 and the lowest in cluster 3 with 4.34. Gomisin A had the highest value in cluster 3 with 4.50 mg/g and the lowest in clusters 1 and 2. Finally, gomisin N was the lowest in cluster 3 with 3.43 and the highest in clusters 1 and 2.

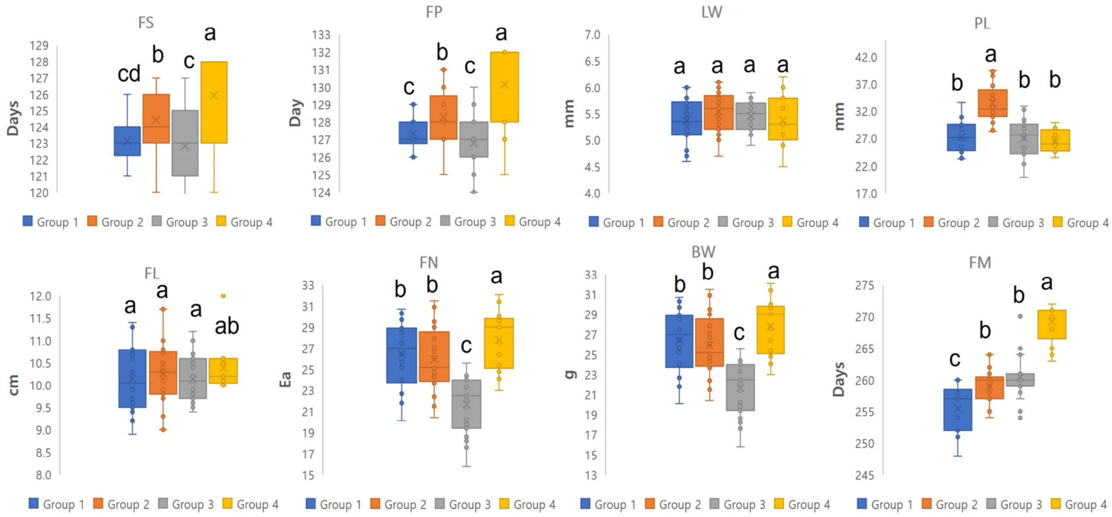

Figure 7. Box plot of agronomical traits in four clusters of 96 *Schisandra chinensis* accessions. FS; start flowering (days to flowering was calculated from 1 January to the day 10% of flowering), FP; flowering period (days to 50% flowering was calculated from 1 January to the day 50% of flowering), LW; leaf width, PL; peduncle length, FL; fruit length, FN; fruit number, BW; bunch weight, FM; fruit maturity (cumulative days after 1 January). LSD is the least square difference between clusters in five different environments, and different characters on bars indicate 5% significance level.

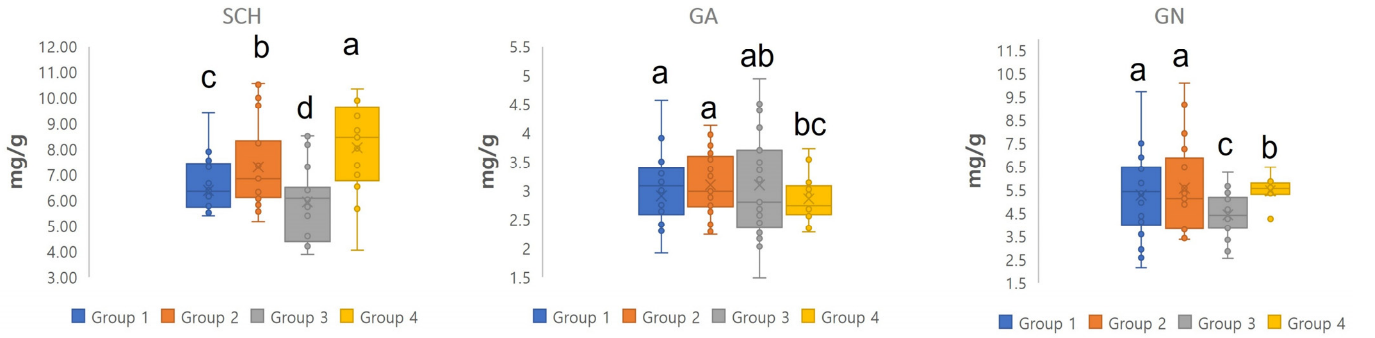

Figure 8. Box plot of lignan content of four clusters of 96 *Schisandra chinensis* accessions. SCH; schizandrin, GA; gomisin A, GN; gomisin N. LSD is the least square difference between clusters in five different environments, and different characters on bars indicate 5% significance level.

4. Discussion

Ninety-six *S. chinensis* accessions distributed in South Korea were collected, and seventeen phenotypic traits and their correlation were investigated for two years. Significant variations were observed for all evaluated traits. *S. chinensis* has been mainly used as an herbal medicine in East Asia and is still included in major herbal medicine prescriptions. In addition, the main efficacy of *S. chinensis* has been scientifically proven by recently published papers, and the relationship between functional components of *S. chinensis* and morphological characteristics could lead to the selection of genetic germplasm. Research on *Artemisia annua* L. indicated that the plant's height and stem bottom diameter had the most important positive influence on the leaf artemisinin content and herb yield [32]. By analyzing the correlation between lignan compounds—schizandrin, gomisin A, and gomisin N—with each trait in this study, we confirmed that the principal components displayed a close correlation with the flower (flowering start, flowering period, and peduncle length) and leaf characteristics (leaf width and length) (Table 6). The main results indicated that it should be possible to select *S. chinensis* accessions with highly functional lignan compounds based on a trait linked to the characteristics of the flowering period.

In particular, gomisin A was closely related to early flowering and small leaves (Table 6). On the other hand, gomisin N was positively correlated with later flowering and larger leaves. Thus, it will be necessary to consider flowering time and leaf size for gomisin contents. The correlation of schizandrin with other morphological characteristics was weak, making it difficult to select an elite line with high schizandrin content relying on a morphological trait. According to Choi et al. [33], the lignan content of *S. chinensis* decreases as the harvest time is delayed. Among the lignan components in this study, schizandrin was related to the harvest period, but the other components were not significantly correlated with maturity (Table 6). Therefore, the factors affecting the *S. chinensis* lignans seem to be genetic rather than due to cultivation methods or the environment. Kim et al. [34] reported the variation in fruit composition of 23 *S. chinensis* accessions and its correlation with fruit weight. As a result, the content in gomisin N did not display any differences across the years. However, our results indicated a variation in gomisin N content depending on the year. In addition, Kim et al. [34] found that schizandrin, gomisin A, and gomisin N contents negatively correlated with fruit weight. This study revealed that gomisin A had a negative correlation with bunch weight, consistent with previous results (Table 6). Regarding gomisin N, a positive correlation with fruit weight was observed in our study. In addition, gomisin N and A are negatively correlated with each other.

In the cluster analysis, RSQ/(1-RSQ) values had the most significant effect on cluster classification, with fruit maturity having the most influence, followed by flowering period traits, such as peduncle length and flowering period and start (Table 9). Flowering traits were important in PCA, but fruit traits were essential in cluster analysis. Therefore, flowering and harvesting are the most important characteristics of *S. chinensis* and should be used to classify *S. chinensis*. Cluster 1 displayed early flowering and harvesting periods, while cluster 4 displayed late ones. When selecting accessions with high gomisin N content, early flowering and maturity were good indicators because cluster 1 displayed high levels of gomisin N, early flowering, and high maturity (Figures 6 and 7). Cluster 2 displayed the highest peduncle length associated with a high level of gomisin A, indicating that those two traits were correlated. In addition, cluster 2 had a higher gomisin N content than clusters 3 and 4 (Figure 7). Through PCA and cluster analyses, the 96 *Schisandra* accessions were compressed into four clusters. This result is expected to help select desirable accessions by understanding the characteristics of each group.

In this study, the basic characteristics and correlations of *Schisandra* were found through the characterization and statistical analysis of 96 *Schisandra* native to South Korea. Understanding the correlation between the agricultural traits of *Schisandra* will make it possible to obtain high-quality plants by early selection. Selecting plants with early flowering time and large peduncle length, correlated with high lignan contents, will improve selection efficiency. In addition, these selected traits can be valuable indicators for selecting accessions in breeding programs, thus reducing the effort required to investigate traits. *S. chinensis* is a perennial medicinal fruit tree that has not been studied much due to its long cropping period and limited resource collection. In addition, there is little information on self-incompatibility in *S. chinensis*, which may increase the efficiency of combining ability in breeding programs. More studies are expected to be conducted, and various roles for *S. chinensis* in industrialization are expected to come to light.

5. Conclusions

In the current study, 96 *S. chinensis* accessions distributed around South Korea were collected, and 17 traits and their correlation were investigated for two years. The relationship between the major agricultural characteristics of *Schisandra* and the major lignan components was investigated. As a result, a significant correlation between phenotypic traits and lignan components was observed. The 13 traits of the 96 accessions were grouped into four clusters based on their characteristics. Flowering and fruit maturity traits were the principal components used to group and compress *S. chinensis* accessions.

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